CONDITIONED TEMPERATURE RESPONSES TO MORPHINE

Roelof Eikelboom

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the degree of Master of Arts at
Concordia University
Montréal, Québec, Canada

September, 1977

ABSTRACT

CONDITIONED TEMPERATURE RESPONSES TO MORPHINE

Roelof Eikelboom

The conditioning of temperature responses using an injection of morphine sulphate as the unconditioned stimulus was studied in 30 male Wistar rats. Three groups of animals received daily i.p. injections of either 5, 25, or an increasing dose to 200 mg/kg morphine; a fourth group received saline injections throughout. Rectal temperature was measured in three different environments five times during the days in a neutral environment, the home-cage; in a pre-injection environment, in which animals were placed for a period before the daily injection; and in a post-injection environment, in which animals remained after the injection. Conditioning trials were followed by a period of abstinence from morphine. Tests for conditioned effects were carried out both during conditioning, when morphine was being administered daily and after the period of abstinence. During the period of daily morphine administration, animals in the morphine groups, when compared to the saline control animals showed a conditioned anticipatory hypothermia in the pre-injection environment and was opposite in direction to the unconditioned hyperthermia to morphine. In contrast, in the post-injection environment, animals in the morphine groups showed a conditioned hyperthermia when tested after the period of abstinence. These results suggest a complex interaction between the conditioned and unconditioned temperature responses to morphine.

Acknowledgements

The author is indebted to several people without whose help this thesis would not have been completed, Dr. J. Stewart, my thesis supervisor, Janney Eikelboom, my wife, Mary Langendyk, Dr. P. Seraganian, and Diane Cygan. They know the contributions they made and words cannot express my sincere thanks.

Table of Contents

List of Tables

List of Figures

Pavlov (1927) was the first to report the use of morphine as an unconditioned stimulus in a study of classical conditioning. Morphine, in the dog, elicits an unconditioned salivary response. If the morphine is preceded by a conditioned stimulus, a tone or even the injection ritual, after several pairings presentation of the conditioned stimulus alone results in conditioned salivation mimicking the unconditioned response. The classical conditioning procedure seems straightforward. Conceptually, Pavlov considered there to be little difference in the conditioning process whether food, acid or morphine was used as the unconditioned stimulus, and, for many years, little further work using morphine was done.

Recently evidence has been presented that raises questions about the adequacy of conceptualizing the conditioned response as one that simply mimics the unconditioned response. While there is evidence that conditioned responses can mimic morphine's unconditioned response as in salivary conditioning (Pavlov, 1927, p. 35), there is also evidence that the conditioned response can be in opposition to morphine's effects, as demonstrated when Siegel (1975) found that tolerance of the analgesic action of morphine was due to a conditioned compensatory hyperalgesia. Similarly, stimuli that have been paired with morphine have been reported capable of preventing the occurrence of withdrawal symptoms (Roffman, Reddy & Lal, 1973),

while in other studies, stimuli that precede morphine administration are later able to produce withdrawal-like symptoms (Wikler & Pescor, 1967). These findings suggest that a detailed examination of the parameters used in such studies and the particular responses studied will be necessary for an understanding of the nature of classical conditioning with morphine.

The early classical conditioning studies using morphine centered almost exclusively on its salivary effects. Several studies were carried out that demonstrated that the phenomenon was easy to elicit, and that it obeyed the laws of conditioning established using more conventional unconditioned stimuli. Collins and Tatum (1925) found that the conditioned effect took about a week of daily sessions to develop, and that it was still present after three to four months. Kleitman and Crisler (1927), in a detailed study, found that the conditioned effect developed rapidly in the first few trials and then followed a negatively accelerating function. The effect extinguished at different rates in different animals, but reacquisition was always faster than the original acquisition. They also reported that partial reinforcement had a detrimental effect on conditioning.

While these studies were of some interest at the time, the use of salivation as the sole unconditioned response may have led to certain assumptions that have only begun to be apparent in recent years. The unconditioned response to morphine

is usually profuse salivation. When no salivation occurs in response to the conditioned stimulus, conditioning is said not to have occurred. However, there is no reason why a "decrease" in salivation could not be considered a conditioned effect. It is difficult however to demonstrate a conditioned decrease in salivation below the floor of "no salivation". In most other physiological systems a "response" can be either positive or negative; heart rate can either increase or decrease from the baseline rate, body temperature can either rise or fall. The fact that in salivary conditioning, the conditioned response is found to "mimic" or to be in the same direction as the unconditioned response, may only reflect the fact that no salivation would be taken to mean no conditioning had occurred. In other response systems a conditioned response that differed in direction from the unconditioned response would be more readily apparent, there being no floor. For this reason, it appeared that a detailed study of morphine conditioning should be done using another physiological system. In the present study temperature response to morphine in the rat was chosen as the response.

Physiological Effects of Morphine

Before proceeding further with an analysis of the conditioned response to morphine the unconditioned responses to a morphine injection should be discussed. The effects of morphine

on any physiological system can best be described under four separate headings; the initial or acute effects, the chronic effects, the effects on morphine termination, that is, the withdrawal effects, and finally, the effects of the morphine antagonists.

The initial effects of morphine on most physiological systems seem to be not only dose related, but also biphasic in nature. In general, small doses of morphine can be said to produce excitatory effects, while larger doses cause biphasic effects consisting first of a depressant phase and then of an excitatory phase (Domino, Vasko & Wilson, 1976; Seevers & Deneau, 1963). The dose that marks the difference between a small dose and a large dose, varies with the response under study, and depends on factors such as the environmental conditions (Paolino & Bernard, 1968) and the route of administration (Lotti, Lomax & George, 1965). The time that any effect lasts appears to increase with dose, but is generally in the range of several hours. Morphine itself does not accumulate in body tissue and is largely gone from the system within 24 hours (Jaffe & Martin, 1975). The dose related biphasic pattern of morphine's effects have been found when measuring general activity (Babbini & Davis, 1972), temperature (Gunne, 1960), and various other physiological responses (Domino et al, 1976). Similarly, rates of bar pressing for food or for electrical stimulation of the brain are first depressed, and then elevated,

by large doses of morphine, but are only elevated by small doses (Babbini, Gaiardi & Bartoletti, 1976; Lorens & Mitchell, 1973).

When considering the effects of repeated or chronic administrations of morphine on a physiological system, two problems should be noted. First, the conditions under which repeated injections and tests for morphine effects are made are largely the same from occasion to occasion. Thus, regardless of the experimenter's intent, the conditions prevail for obtaining conditioned effects. Second, it might be expected that the effectiveness of a drug will decrease with repeated administrations. This tolerance or decreasing responsiveness of a system to repeated doses of a drug, has been found to occur for only some of the effects of morphine. There is considerable evidence that, while morphine depressant effects tolerate rapidly, its excitatory effects do not (Seevers & Deneau, 1963). However, it does appear that, with repeated injections of large doses, the excitatory effects move forward in time. This change over repeated injections has been reported for general activity and metabolic rate (Martin, Wikler, Eades & Pescor, 1963), temperature (Gunne, 1960), electrical self-stimulation (Bush, Bush, Miller & Reid, 1976) as well as the more basic physiological measures (reviewed by Seevers & Deneau, 1963). Furthermore the effects of a frequently repeated large dose of morphine seem to be similar to the effects of an initial small dose. While there

are a few reports of tolerance to the excitatory effects of small doses of morphine (Glick & Rapaport, 1974; Siegel, 1976, Note 1), the majority of studies have failed to find evidence of tolerance to effects of small doses of morphine (Babbini & Davis, 1972; Esposito & Kornetsky, 1977; Lal, Miksic & Smith, 1976; Lorens & Mitchell, 1973).

One of the more important properties of morphine is its analgesic effect. The analgesic effect measured in laboratory animals, using heat, pressure, or electric shock, tolerates rapidly. If a constant dose is chronically administered, there is evidence not only for tolerance of this analgesic effect, but, in fact, a hyperalgesic response has been observed (Kayen, Woods & Mitchell, 1971). This suggests the possibility that even the analgesic effects of morphine may follow the pattern of its other effects; with repeated injections the initial depressant phase disappears and is gradually replaced by an excitatory response.

Third, termination of morphine administration, especially after chronic large doses, results in a constellation of rebound responses that have been labeled withdrawal symptoms. A dose that produces withdrawal symptoms is said to cause dependence. Martin et al (1963) report a two-phased withdrawal after drug administration in the rat. The first phase lasts a matter of days and is characterized by weight loss, hypothermia, wet dog shakes, and increased activity. The

second phase lasts several months and is characterized by rapid weight gain, hyperthermia, some residual wet dog shakes, increased metabolic rate and increased activity. A similar biphasic withdrawal has been reported in humans (Jaffe, 1975).

Lastly, it is necessary to mention the morphine antagonists, as they have been used in several conditioning studies to elicit the withdrawal symptoms, i.e., as unconditioned stimuli. The antagonists are chemicals that are structurally sufficiently similar to morphine that they can block most if not all of morphine's effects. They range from partial antagonists, such as nalorphine, that have some morphine-like properties, to pure antagonists, such as naloxone, that seem to have no effects in themselves (Martin, 1967). Their most striking property is the ability to produce immediate withdrawal symptoms in dependent animals. Pure antagonists also block most if not all of the initial effects of morphine (for a review see Jaffe & Martin, 1975).

Conditioning of Morphine Effects

Given the complexity of the unconditioned response to morphine, it is not surprising that diverse conditioned effects have been observed. There is, however, a point that should be clarified before discussing the conditioned effects. As conditioning is rarely evident after one trial, but rather is measurable only after many pairings of the conditioned stimulus

and the morphine, it really is not possible to describe conditioning to the acute effects of morphine. It is only the chronic effects of morphine that have been studied. Moreover, as was mentioned previously, in most studies of the chronic physiological or unconditioned effects of morphine, the conditions are similar to those in a classical conditioning study. The injection ritual can be viewed as a conditioned stimulus that is always followed by the morphine, the unconditioned stimulus. In addition, there are, frequently, other cues such as time of day or place of injection that can act as conditioned stimuli. Thus, it may be that only the initial effects of morphine can be said to be purely physiological. After several injections the "morphine" effect may be due both to physiological and conditioned effects. Thus, while repeated pairings of the conditioned stimulus with an injection of morphine are necessary for conditioning, it is only the effect of the first injection of morphine that constitutes the unconditioned response.

Tolerance is a term that is used to describe changes in morphine effects after repeated injections. Siegel (1975) has suggested that tolerance, in itself, is due to a classically conditioned response that differs in direction from the direct action of morphine and, in that sense, is antagonistic or compensatory. In studying the analgesic effects of morphine, Siegel (1975, 1976) found evidence that tolerance is a learned situation-specific effect. Pain sensitivity can be measured by

placing a rat on a hot-plate and measuring the latency to paw-lick. If morphine is administered to rats, its effect is to increase the paw-lick latency, an indicator of its analgesic effect. With repeated injections this effect disappears, an example of tolerance. If other rats are given similar injections of morphine, but in an environment distinctly different from the test environment, they show no tolerance when first tested on the hot-plate. Moreover, if animals are injected in the test environment, but are given an analgesic test only once, on the last day in that same environment, they do show tolerance. Thus it appears that tolerance observed in these animals is a learned situation-specific response. Siegel found, in addition, that tolerant animals when tested with saline showed hyperalgesic responses (Siegel, 1975). It was this finding that led to the suggestion that tolerance is a conditioned compensatory response to morphine's initial effects. Since the original studies, Siegel (1977) has gone on to show that the conditioned effect can be manipulated by variables that effect other classically conditioned responses; he has observed extinction, latent inhibition, and a decrement due to partial reinforcement.

Some of the conditioned effects of morphine, however, appear to mimic the original response to morphine. It is interesting to note that these responses, more often than not, are ones that have been reported not to tolerate. These are the so-called excitatory effects, in that they are apparent as

increases in the various physiological measures. One response already discussed is the conditioned salivary response. Increases in general activity caused by small doses of morphine can be conditioned (Kamat, Dutta & Pradhan, 1974), as can small-dose temperature increases (Miksic, Smith, Numan & Lal, 1975).

Schuster and Woods (1968) were able to show classical conditioned effects while studying animals self-administering morphine. Each time the animals bar-pressed a conditioned stimulus, a light, was paired with the morphine. During extinction the light, the conditioned stimulus, was presented after each bar press for half the animals. Animals presented the light, maintained bar pressing longer than animals not presented with the light. Thus, it appeared that, through classical conditioning, the light had taken on some of the positive properties of morphine and now acted as a conditioned reinforcer. Similarly, animals that have learned to drink a morphine solution will afterwards drink a quinine solution (a solution that tastes similar to a morphine solution), even though control rats will not (Kumar, 1972). In this case the bitter taste has, through conditioning, taken on some of the positive properties of morphine.

Another effect of morphine is its ability to prevent withdrawal symptoms; there is evidence that this effect may be conditionable. Tye and Iversen (1975) had rats that were responding on a VI schedule for food reinforcement, and that were

also being administered, twice daily, gradually increasing doses of morphine, paired with a distinctive conditioned stimulus, a 10-minute tone presentation. During testing, morphine administration was terminated for all animals, but half the animals continued to receive the conditioned stimulus. They found that in the group without the conditioned stimulus, the response rate for food dropped drastically for the first two days and then gradually returned to normal. In the group still receiving the conditioned stimulus the withdrawal effect was not evident, and the response rate remained constant throughout this period. Thus, the conditioned stimulus that had been paired with morphine was able to prevent the withdrawal effects. However, this same conditioned stimulus was unable to prevent the withdrawal response precipitated by an injection of naloxone.

Because of its potential relevance for drug-taking behavior, several researchers have been interested in the conditioning of withdrawal effects. Wikler and Pescor (1967) made rats dependent by giving two gradually increasing injections per day. They then gave the rat only one injection per day (200 mg/kg) and 16 hours after the injection put the animals in a distinctive environment, where the animals exhibited primary withdrawal symptoms. After 7½ hours in this environment the animals were removed and received, within the next half hour, their daily injection of morphine. After several pairings morphine administration was terminated and animals were tested

for wet dog shakes, in the home-cage or the withdrawal environment. They found more wet-dog shakes in the withdrawal environment than the home-cage up to 72 days after morphine termination. They concluded that withdrawal symptoms could be paired with a particular environment. Trost (1973) reported similar success in conditioning withdrawal symptoms using wheel running as a dependent measure.

Goldberg, Woods and Schuster (1969) repeatedly paired a conditioned stimulus with nalorphine injections in monkeys that were self-administering morphine. Nalorphine alone, a morphine antagonist, resulted in increased morphine self-administration. After several pairings the CS alone was able to produce a similar rise in morphine's self-administration. In another experiment, with dependent animals that were bar pressing for food, a conditioned stimulus previously paired with nalorphine suppressed responding in the same way nalorphine did (Goldberg & Schuster, 1967).

The idea that withdrawal symptoms could become conditioned to the environment in which they occurred has been tested recently using heroin addicts on a methadone maintenance program. O'Brien, Testa, O'Brien, Brady & Wells (1977) repeatedly paired a distinctive environment with small doses of naloxone, producing mild withdrawal like symptoms, in eight human heroin addicts. Afterwards, saline, given in that environment, was able to cause similar withdrawal effects.

In summary, the studies reviewed in this section have revealed evidence for a number of divergent conditioned effects of morphine. It has been shown, for example, that conditioned responses may account for the tolerance to morphine, the conditioned response being in opposition to the morphine effect, and, as it gets stronger, causing an apparent weakening of the initial morphine effect. Other conditioned responses to morphine have been found to mimic the initial morphine effects. Why conditioned responses mimic some of the initial effects of morphine and oppose other morphine effects, causing tolerance, is not clear at this time. Because of the biphasic nature of the response to high doses of morphine it is often difficult to determine whether the conditioned effect is in opposition to the initial depressant effect or one that mimics the excitatory phase of the morphine effect. This dilemma may be resolved by comparing the conditioned responses obtained with large doses of morphine to those obtained with small doses, ones that usually have only the excitatory effects.

The finding that a stimulus that has been paired with the occurrence of withdrawal symptoms can elicit withdrawal-like effects, both in animals still receiving morphine, and in animals long since withdrawn from morphine, is, for different reasons, difficult to interpret. It should be noted, however, that in those studies in which morphine withdrawal is paired with a conditioned stimulus (in order to elicit conditioned

withdrawal symptoms), the withdrawal state is reliably followed by a morphine injection. When naloxone has been used to elicit withdrawal symptoms in animals already under the influence of morphine, naloxone's effects being short lasting, are soon followed by relief from withdrawal, or the morphine "state". It is not clear, therefore, what is being conditioned, the current withdrawal symptoms, or a preparatory response to the oncoming morphine. By looking for evidence of conditioned effects in the period prior to each morphine injection, in animals receiving either large or small doses of the drug, it may be possible to tease out the nature of the conditioned response.

Another problem that make the results of conditioning studies difficult to interpret is that these divergent conditioned effects have been found in different studies using various unconditioned responses, different doses of morphine, a diversity of conditioned stimuli, and assorted time parameters. The present experiment, therefore, was designed to overcome these difficulties. Thermoregulation seemed, for several reasons, an ideal response system to study. Unlike salivation, body temperature can rise or fall creating the possibility for the conditioned response to mimic or oppose the unconditioned morphine temperature response. Repeated temperature measures are easily taken permitting the measurement of temperature in animals at various times both before and after morphine administration. Further, many animals can be studied simultaneously making the

comparison of several morphine doses possible.

There are several studies in which particular aspects of the conditioned temperature response to morphine has been examined (Miksic et al, 1975; Roffman et al, 1973; Siegel, 1976, Note 1). They have generally suffered from the flaws mentioned above; single doses of morphine have usually been examined within different experimental procedures. It is, therefore, not surprising that divergent results are reported (Miksic et al, 1975; Siegel, 1976, Note 1). Before discussing in more detail these and other studies, it would be advisable to review the unconditioned temperature response to morphine. This shall be done, as was done for the unconditioned effects in general, by considering the initial effects, the chronic effects, the effects of termination of morphine administration, and finally, the effects of the morphine antagonists.

Temperature Effects of Morphine

Initially, in the rat, the effects of morphine on temperature, like its other effects, are dose dependent. Small doses produce a hyperthermic response, whereas large doses produce the usual biphasic effect, first hypothermia and then hyperthermia (Cox, Ary, Chesarek & Lomax, 1976; Gunne, 1960; Herrmann, 1942; Lotti et al, 1965). The length of time that body temperature is affected by morphine increases with dose and ranges from four to eight hours (Gunne, 1960; Lotti et al, 1965). The dose

of morphine at which the change from a pure hyperthermic to the biphasic response occurs has been shown to depend both on the route of administration and on ambient temperature. Below 30 mg/kg, a subcuteanous injection of morphine causes hyperthermia, above 30 mg/kg, the response is first hypothermic and then hyperthermic (Gunne, 1960). With intravenous or intraperitoneal injections, the critical dose of morphine appears to be around 10 mg/kg (Cox et al, 1976; Lotti et al, 1965). Paolino and Bernard (1968) studied the effects of the ambient temperature on the initial response to morphine. They found that 50 mg/kg of morphine, injected intraperitoneally to rats, produced either a hyperthermic response (in a 30° C room), a hypothermic response (in a 5° C room) or a weak hyperthermic response (in a 24° C room).

As expected, the second and subsequent injections of morphine produce different effects from the first injection. When large doses of morphine are administered chronically, the hypothermic response disappears and is replaced by a hyperthermic response, the peak of which moves forward in time (Cox et al, 1976; Gunne, 1960; Lomax & Kirkpatrick, 1967). Gunne (1960) found that the hyperthermic response to the large dose did not tolerate. In a recent study by Lal et al (1976), in which a small, 10 mg/kg dose of morphine was injected intraperitoneally, it was shown that there was a strong hyperthermic response even after 39 injections. Likewise, Roffman et al (1973) and Martin

et al (1963) have found that even in dependent rats each injection of morphine causes the expected hyperthermia. However, Siegel (1976, Note 1) has presented evidence suggesting that the hyperthermic response to small doses of morphine does tolerate with repeated injections.

In the dependent rat the initial effect of termination of morphine administration has been reported to be a mild hypothermia. The animal's body temperature decreases for about 24 hours, when the temperature is approximately 1.5° C below normal, and then returns to baseline after about 72 hours (Roffman et al, 1973). Martin et al (1963) report that, after the hypothermia, there is a second phase of withdrawal characterized by a hyperthermia that occurs four or five days after morphine termination and lasts approximately three months.

The hypothermic response to the initial dose of morphine can be blocked by the morphine antagonist nalorphine (Lotti et al, 1965). This blockade by nalorphine of morphine effects also prevents the development of tolerance, that is, the second injection still causes a strong hypothermic response (Lomax & Kirkpatrick, 1967). Recently, however, Cox et al (1976) have presented data suggesting that naloxone does not block the hyperthermia produced by small doses of morphine. They compared three groups of rats: one group injected with 0.5 mg/kg of naloxone only, the second with 4 mg/kg of morphine only and the third with both naloxone and morphine. While

animals in the naloxone alone group showed almost no change in body temperature, the third group, receiving both drugs, was actually more hyperthermic than the morphine only group. This, if true, would make hyperthermia one of the few effects of morphine that cannot be blocked by naloxone. There is, however, another possible explanation for these findings. The half-life of naloxone, in the rat, is approximately 20 minutes (Weinstein, Pfeffer & Schor, 1974) and the dose of naloxone given in the Cox et al (1976) study was small. There may not have been enough naloxone to block the morphine for the full duration of its effects. This possibility makes it necessary to study the effects of a larger, and perhaps a repeated dose of naloxone, before it is concluded that the hyperthermic response cannot be blocked by morphine antagonists.

In dependent animals, the morphine antagonists cause immediate onset of withdrawal symptoms, that is, a hypothermic response (Ary, Chesarek, Sorensen and Lomax, 1976; Drawbaugh & Lal, 1974). It should also be noted that Drawbaugh and Lal (1974) have reported that, in the dependent animal, naloxone does block morphine induced hyperthermia, a finding that casts further doubt on the reliability of the Cox et al (1976) report that the initial hyperthermia to small doses of morphine is not blocked by naloxone.

Conditioned Morphine Temperature Effects

There have only been a few studies on the conditioned temperature responses to morphine and, given the complexity of the unconditioned temperature response to morphine it is not surprising that the findings are inconsistent. Most studies used only one dose of morphine; and from one study to the next different control conditions have been used to test for a conditioned effect, making the results difficult to compare. Conditioned effects that mimic (Miksic et al, 1975) and oppose (Siegel, 1967, Note 1) the temperature effects of small doses of morphine have been reported. A discussion of the conditioned temperature effects using small doses of morphine, the conditioned temperature effects observed in dependent animals, and the effects of the morphine antagonists on these conditioned responses follows.

Miksic et al (1975) have reported that a stimulus, a tone in a distinctive environment, repeatedly paired with a daily injection of 20 mg/kg of morphine (route of administration unspecified), elicited hyperthermia, a mimicking of the morphine effect, when presented alone. In their study, each animal served as its own control, and the measure of the hyperthermia was the difference between the temperature measured before presentation of the conditioned stimulus and 30 minutes afterward. Using this design it is not possible to determine whether animals were hypothermic relative to some neutral baseline prior to the presentation of the stimulus predicting morphine and merely

returned to normal after, or whether they were at a normal body temperature before and were hyperthermic after stimulus presentation. This procedural point may be important as Siegel (1976, Note 1) has evidence that the conditioned temperature response to a stimulus paired with a 5 mg/kg subcutaneous injection of morphine is hypothermia if the temperature is compared to that of control group animals that received only saline injections throughout. This is a matter that requires further study.

If stimuli that have been paired with morphine injections can elicit responses that mimic the effects of morphine and produce, for example, hyperthermia, it might be that they can prevent the hypothermia evident in dependent animals after withdrawal of morphine. In fact Roffman et al (1973) had found, prior to the work of Miksic et al (1975) reported above, that if a bell was repeatedly paired with dependence-producing morphine injections (four injections daily between 8 and 22 h increasing to a maintenance dose of 200 mg/kg/day), the bell alone could prevent the hypothermic withdrawal response, and also reverse the hypothermia if it had been allowed to start.

One of the more interesting questions is what effect might morphine antagonists have on these conditioned effects? If the antagonists were found to block the conditioned effects, it would suggest that the brain systems involved in the conditioned responses are similar to those involved in the direct effects of morphine. Lal et al (1976) found that naloxone did

block the effect on body temperature of a conditioned stimulus that had been paired with 20 mg/kg morphine injections. They found that, 5 mg/kg of naloxone would prevent the conditioned hyperthermic response. Furthermore, Drawbaugh and Lal (1974) using dependent animals found that when the conditioned stimulus was followed by an injection of naloxone (instead of the usual morphine) there was a pronounced hypothermic response that was greater than that after naloxone alone (both in animals that received their last morphine injection 24 hours before). This suggests that naloxone has more effect in situations that have been paired with morphine than in neutral situations, and re-opens the question of the direction of the conditioned temperature effects as the conditioned stimulus plus saline can prevent the usual withdrawal hypothermia. If the conditioned stimulus augments the naloxone produced hypothermia it might in itself be evoking a temperature lowering mechanism that is otherwise masked.

In order to evaluate some of these divergent results, an experiment was planned that would allow one to look at several of these conditioned temperature effects under a single set of experimental conditions. Three groups of rats were conditioned using widely differing doses of morphine, and all temperature measures were compared to those found in a fourth saline control group. Body temperature measurements were taken in three different environments: a pre-injection environment, a distinctive room in which animals were placed for two hours

prior to being taken to another room for the morphine injection; a post injection environment, on entry to which the animals were immediately injected, and, therefore, a room in which the effects of morphine would occur; and a neutral environment, the home-cage. Thus it was possible to test for conditioned effects prior to and after the morphine injection. Morphine was administered once every 24 hours to permit discrete conditioning trials. After repeated conditioning trials, various drugs were substituted for the usual morphine: saline, to test for conditioning; a low dose of morphine, to study the interaction, if any, between the conditioned and unconditioned response; and naloxone, to study the effects of an antagonist on the conditioned effects. In order to tease out the "pure" physiological effects of not receiving the usual morphine injection, all "tests" were carried out twice, once in the usual conditioning environment, and again, in the home-cage (the neutral environment). Any difference found between the response of the animals in the two situations could then be interpreted as due to conditioning. After the conditioning trials ended, the animals were housed in their home-cages for several days, until primary withdrawal was completed. They were then tested again for the various conditioned effects. This design permitted tests for conditioned effects during the period when animals were receiving daily administrations of morphine, and, again, during a period when the animals were no longer receiving daily morphine and were past the primary withdrawal period.

Method

Subjects

Thirty-seven male Wistar rats, weighing 150-175 g at arrival, were obtained from Canadian Breeding Farm and housed individually in stainless steel cages (18cm x 25cm x 18cm) for the duration of the study. Purina Lab Chow and water were available to the animals at all times in the home-cage. The animal room was lit from 7:00 h to 21:00 h and was maintained at a constant temperature of 22 ± 1° C. Animals were randomly assigned to one of four groups, differing in the daily dose of morphine they would receive throughout the study: Group 1, n = 8, received 0 mg/kg; Group 2, n = 8, received 5 mg/kg; Group 3, n = 9, received 25 mg/kg; Group 4, n = 12, received increasing doses up to 200 mg/kg.

Seven animals from Groups 3 and 4 died early in the experiment. These animals all died within two hours of receiving an injection of morphine. Five animals died after receiving 25 mg/kg of morphine on either Day 5 or 6 of the experiment, the other two died after a 40 mg/kg and 55 mg/kg injection of morphine on Day 9 and 13 respectively. All data previously collected from these animals were excluded from the various analyses. As a result of these deaths the number of animals in each group for all of the analyses was eight for Groups 1 and 2 and seven for Groups 3 and 4.

Procedure

During the first four weeks after arrival the rats were housed 24 hours per day in their home-cages; from time to time they were weighed and handled. On four alternate days during the third week, rectal temperatures were measured at 9:00, 12:00, 15:00, 18:00, and 21:00 h. These temperature measurements provided a home room baseline and accustomed the animals to the temperature taking procedure.

Temperature measurement: Rectal temperature was measured by means of a small animal probe (Yellow Springs model 402) and a Yellow Springs Tele-Thermometer Model 46 TUC (accuracy = ± .15° C). The rats were placed in a small rectangular trough closed at one end (7cm x 22cm x 8cm) and held down with one hand while the probe was inserted a minimum of 6 cm as recommended by Lomax (1966) for approximately 30 seconds until the temperature reading stabilized. After two or three measurements the rats accepted the procedure with little objection (no biting, squealing or kicking and only minimal struggle against the restraint).

Drugs and injection procedures: Throughout the study all drugs were injected intraperitoneally. All solutions were made up using physiological saline, and, where possible, the drug concentration was adjusted so that the volume injected was 1 ml/kg. Morphine sulfate was used throughout and all doses refer to the salt. For Group 4, at the higher doses it was necessary to increase the volume injected to 3 ml/kg. Naloxone

hydrochloride (Endo) was always injected at a dose of 2 mg/kg.

- Daily routine: Four weeks after the rats arrived in the laboratory the experiment proper was started. With the exception of the home-cage days (see below), the daily routine was the same for all animals throughout the entire study. Essentially it consisted of taking the animals from the home room to a distinctive pre-injection environment where they were immediately injected with their daily dose of morphine. Thus it was possible to take temperature measurement in the home room, in an environment predictive of morphine, but in which morphine was never given, and in an environment where the effects of morphine occurred. The details of the routine were as follows.

At 9:00 h all animals were weighed and their temperatures were measured. One hour later, at 10:00 h, the rats were placed, in groups of three or four, in a carrying cage and taken to a pre-injection room, where they were individually housed in wooden boxes (17cm x 28cm x 13cm) with wire tops and wood shavings on the floor. This room was dimly lit, was quiet, had an oil of cloves odour cue, and was maintained at a temperature of 22 ± 1° C. At 11:00 h all animals had their temperature measured in this room. At 12:00 h, following the two-hour period in the pre-injection room, the animals were moved in their boxes to a second room (the post-injection room) where they were immediately given an injection of the appropriate drug. The post-injection room was brightly lit, had a continuous 12 db white noise back-

ground, had no odour cue, and was maintained at a temperature of 23 ± 1° C. At 12:45 h and again at 14:45 h (45 and 135 minutes after the drug injection) the rectal temperatures were measured in this room. After three hours in the pre-injection room, 15:00 h, animals were returned, in groups of three or four, to their home-cage. At 18:00 h temperature was measured for the last time. At no time was food or water available in either the pre-injection or post-injection room. The animals were always handled and treated in the same order; keeping the time between events the same for all animals.

The daily routine described above was followed every day with the exception of the home-cage days. On these days the animals remained housed in their home-cages throughout the day, but were weighed and had their temperatures taken at the usual times. On some of these days the animals were injected as usual at 12:00 h, but on others no injection was given (for a summary see Table 1).

Design over days: The experiment proper consisted of seven phases; a baseline saline period (Days 1-4), a naloxone morphine day (Day 5), a conditioning period (Days 6-32), a "morphine test" period (Days 33-85), an abstinence period (Days 86-94 and two "post-morphine test" periods (Days 95-100 and Days 120-122).

Days 1 to 4 were baseline days. On these days the animals were taken through the normal daily routine; the injection given to all animals at 12:00 h was physiological saline.

TABLE 1

Routine on normal conditioning days and on home-cage days.

Time	Normal day	Home-cage day
9:00	Weighing Temperature reading	Weighing Temperature reading
10:00	Move to pre-injection room	
11:00	Temperature reading	Temperature reading
12:00	Move to post-injection room Injection	(Injection)
12:45	Temperature reading	Temperature reading
14:15	Temperature reading	Temperature reading
15:00	Return to home-cage	
18:00	Temperature reading	Temperature reading

Day 5 was the naloxone morphine day. On this day all animals followed the normal routine but were given two injections at 12:00 h, spaced approximately 5 minutes apart. Animals in Group 1 were first injected with naloxone and then with saline. Group 2 animals were injected with naloxone followed by an injection of 5 mg/kg of morphine. Animals in Group 3 were injected with naloxone and then with 25 mg/kg of morphine. Animals in Group 4 first received an injection of saline, then 25 mg/kg of morphine.

Days 6 to 32 were conditioning days in which the normal daily routine was always followed. Animals in Groups 1, 2, and 3 received daily injections of 0 (saline), 5, and 25 mg/kg of morphine, respectively, throughout this period; animals in Group 4 were injected with increasing doses of morphine starting with 25 mg/kg of morphine on Day 6 and increasing to 200 mg/kg by Day 32 (see Table 2 for details). The animals in Group 1 were injected with a volume of saline equivalent to that given to animals in Group 4.

Days 33 to 85 were, with the exception of the "test" days, maintenance days in which animals in Groups 1, 2, 3, and 4 received their usual injections of morphine, 0, 5, 25, and 200 mg/kg respectively. The "test" days should be considered in pairs, on the first of the two days, the normal daily routine was followed; on the second, the animals remained in the home-cage. On Day 37, the first of the "test" days, animals were taken

TABLE 2

Morphine dose, concentration, and volume given to animals in Group 4 on Days 6 to 32.

Days	Dose mg/kg morphine sulfate	Concentration mg/ml	Volume mg/kg saline
6, 7	25	25	1
8, 9, 10	40	40	1
11, 12, 13	55	55	1
14, 15, 16	70	70	1
17, 18, 19	85	56.6	1.5
20, 21, 22	100	66.6	1.5
23, 24, 25	120	60	2
26, 27, 28	140	70	2
29, 30, 31	170	56.6	3
32	200	66.6	3

through the normal routine, but were all injected with 1 ml/kg physiological saline. On Day 43, the paired day, animals remained in the home-cages and received no injections. On Day 51, a routine day, all animals were injected with naloxone; a similar injection was given on Day 57 in the home-cage. On Day 65, a routine day, and again on Day 71, a home-cage day, all animals were injected with 5 mg/kg of morphine. On Day 79, a routine day, saline injections were given to all animals, and on Day 85, the paired home-cage day, no injections were given.

Days 86 to 94 were home-cage days during which no injections were given, but temperatures were measured. Days 95 to 99 were routine days, but ones on which morphine was not given. On Days 95 and 96 all animals received saline, on Day 97 naloxone, and on Days 98 and 99 saline. Day 100 was a home-cage day during which the animals were not injected.

From Day 101 to 119 the animals were left in their cages. The animals were untouched during this period; neither temperature nor weight was measured, nor were injections given during this period.

Days 120 to 122 were the last three days of the experiment and consisted of a normal routine day (Day 121) when all the animals were injected with saline, and two home-cage days (Days 120 and 122) when no injections were given.

Table 3 contains a summary of the design, over days, of the experiment.

TABLE 3

A summary over days of the design of the experiment.

Days		
1 - 4	Baseline	Normal routine with saline injections
6 - 36	Conditioning	Normal routine with morphine injections
37 - 85	Conditioning days interspaced with:	
37	Saline test day	Normal routine
43	Home-cage day	No injections
51	Naloxone test day	Normal routine
57	Home-cage day	Naloxone injections
65	Morphine test day	Normal routine
71	Home-cage day	Morphine injections
79	Saline test day	Normal routine
85	Home-cage day	No injections
85 - 94	Abstinence	Home-cage days with no injections
95 - 96	Saline test	Normal routine with saline injections
97	Naloxone test	Normal routine with naloxone injections
98 -99	Saline test	Normal routine with saline injections
100	Home-cage	No injections
120	Home-cage	No injections
121	Saline test	Normal routine with saline injections
122	Home-cage	No injections

Results

Stated briefly, the analyses of the data from the experiment as a whole revealed two different conditioned temperature responses; one evident in the period just prior to the daily injection of morphine, the other occurring in the period after the daily injection. The pre-injection response was a conditioned lowering of body temperature, relative to the control group, while the post-injection response was a conditioned increase in temperature, relative to the control group. Note that the pre-injection conditioned response was in the direction opposite to the unconditioned hyperthermia while the post-injection conditioned response mimicked the unconditioned response. During the period of daily morphine injections, the post-injection conditioned response appeared to be masked by the pre-injection conditioned lowering of body temperature. In the period after abstinence from morphine, however, the post-injection conditioned rise in body temperature was clearly evident.

In the sections that follow, the analyses of the data upon which these general statements are based will be considered in detail. For convenience these have been divided into four subsections: baseline and unconditioned response data, the pre-injection conditioned response, the post-injection conditioned response, and, finally, the tests using naloxone and 5 mg/kg of morphine.

Baseline Temperature Data and the Unconditioned Response

Pre-experiment home-cage baseline: Before the experiment proper started, a series of temperature measurements were taken in the home room. The data were analysed using a Group x Time x Day analysis of variance (Appendix A, Table 1). As expected, there was no significant Group effect, nor were there any significant Group interactions. However, the Time, Days, and Time x Day effects were all significant. Further analysis using Scheffé tests revealed that animals had a higher temperature at 21 h than at any other time during the day ($p < .05$). The other times did not differ significantly from each other. Further none of the individual comparisons comprising the Day or Time x Day effects reached significance using the Scheffé test.

Conditioned environment baseline: On Days 1 to 4 of the experiment, all animals were taken through the daily routine; they were placed in the two conditioning environments at the appropriate times and were given injections of saline. Temperature was measured at each of the standard times. A Group x Time x Day analysis of variance of the temperature reading revealed that only the Time, Group x Time, and Time x Day effects were significant (Appendix A, Table 2). Figure 1 shows the mean temperature for each of the five times, for all groups and days combined. It can be seen that body temperature rose, relative to

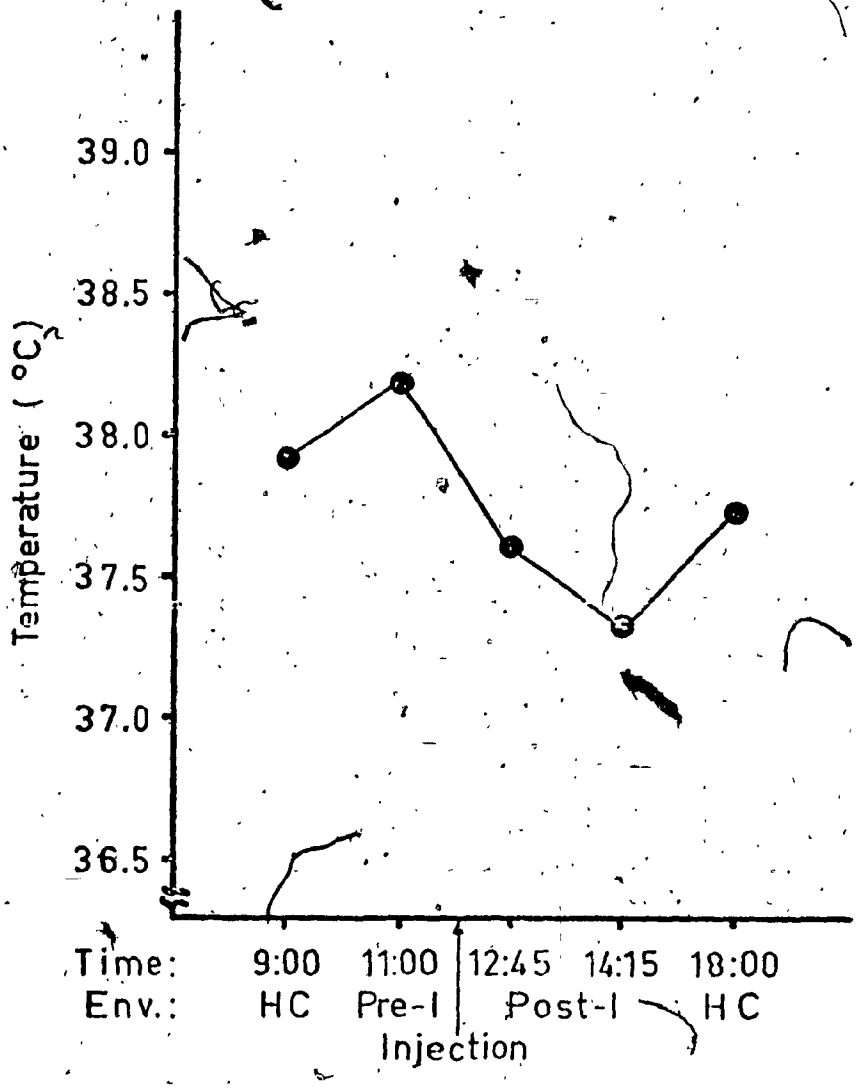

Figure 1: Mean saline baseline body temperature of all animals averaged over Days 1 to 4 at various times of the day in the home-cage (HC), the pre-injection environment (Pre-1), and in the post-injection environment (Post-1).

what it was in the home-cage, in the pre-injection environment, and fell slightly in the post-injection environment. Because of the significance of the Time effects, individual Group x Day analyses of variance were run for each time (Appendix A, Tables 3 to 7). The results for each time were consistent; there were no significant Group or Group x Day effects, but in each case, the Day effect was significant. This suggests that though there were daily variations in the animals' body temperatures, these daily effects were consistent across groups. As will be evident these daily variations in body temperature continued to occur throughout the experiment.

Home-cage temperature at 9 h during the period of conditioning: Each day at 9 h the temperature of all the animals was measured in the home cage. Two analyses of variance of these 9 h readings were done to test for group differences; in the first, readings on Days 6 to 9 were considered; in the second, readings taken every seventh day, from Day 7 to 84, were analysed. The Group x Day analysis for Days 6 to 9 revealed no significant effects (Appendix A, Table 8). The Group x Days analysis for every seventh day from Day 7 to 84 revealed only a significant Days effect (Appendix A, Table 9). Because there was no evidence that the temperatures of the groups differed at the 9 h reading, the saline group was considered to be an appropriate control group for assessing differences between treatments at other times of the day. Further, the lack of group differences at 9 h suggests

that there were no physiological effects on body temperature of the previous days' morphine administration evident at this time.

Morphine naloxone day: On Day 5, at 12 h, all animals were given two injections to determine the effect of naloxone on the initial hyperthermic response to morphine. Two one-way analyses of variance, one for the 12:45 h and one for the 14:15 h temperature readings, revealed a significant Group effect in each case, $F(3, 26) = 14.87$, $p < .001$ and $F(3, 26) = 10.35$, $p < .001$. Further analysis using the Scheffé test revealed that in neither case was the mean body temperature of animals in Group 1 (the naloxone alone group) different from those in Group 2 (the naloxone and 5 mg/kg of morphine group) (at 12:45 h $F(3; 26) = 2.84$, $p > .25$; at 14:15 h $F(3, 26) = 2.76$, $p > .25$). At neither time did animals in Group 3 (naloxone and 25 mg/kg of morphine) and Group 4 (25 mg/kg of morphine) differ but at both times animals in Groups 3 and 4 were significantly warmer than animals in Group 1 ($p < .05$). Thus while 2 mg/kg of naloxone was able to block the hyperthermic response to 5 mg/kg of morphine, it had little effect on the larger dose.

Morphine effects: Separate analyses of variance were carried out for the two temperature measurements taken in the post-injection environment, at 12:45 h, and at 14:15 h. The initial effects of morphine were studied by doing a Group x Day analysis for Days 6 to 9. The analysis of the 12:45 h temperature showed a significant Group effect and a Group x Day interaction

(Appendix A, Table 10). The mean temperature of animals in each group at 12:45 h for Days 6 to 9 is shown in Figure 2. It seems evident that, overall, the three morphine groups did not differ from each other, but that they were all strongly hyperthermic relative to Group 1, the saline group (Scheffé, $p < .05$). Comparison made on individual days, however, showed that on Days 8 and 9, Group 4, the ascending dose group, differed significantly from Groups 2 and 3. Note that it was on Day 8 that the morphine dose for Group 4 was increased for the first time, from 25 to 40 mg/kg. At 14:15 h only the Group effect was significant (Appendix A, Table 11) with the three morphine groups being hyperthermic relative to Group 1, the saline group ($p < .05$). Groups 3 and 4 were not different from each other, but both had a significantly higher temperature than Group 2 ($p < .05$).

The long-term changes in the effects of morphine were assessed by comparing the groups every seventh day over the period of morphine administration. Two Group x Day analyses were done, one at each of the two post-injection times (12:45 h and 14:15 h). In both cases all the main effects and interactions reached significant levels (Appendix A, Tables 12 and 13). Comparison of the Group means, however, revealed little evidence for a trend across days. Thus two analyses comparing the first three days (7, 14, and 21) to the last three days (70, 77, and 84) were done using a Group x Block x Day design (Appendix A, Tables 14 and 15). At neither time, 12:45 h nor 14:45 h, was the Group x

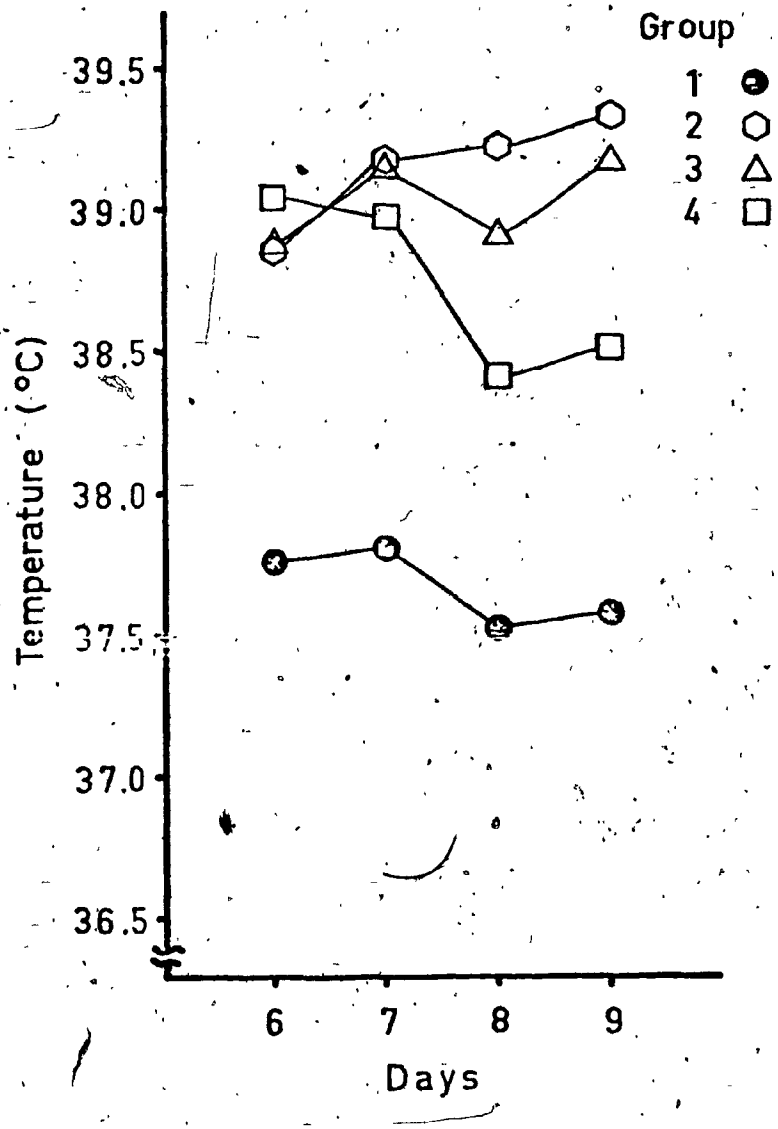

Figure 2: Mean body temperature of animals in the four groups at 12:45 h in the post-injection environment, 45 minutes after morphine administration, for Days 6 to 9.

Block interaction significant, suggesting that there was no consistent change or trend in the effectiveness of morphine over the duration of the experiment.

Home-cage temperature at 18 h during the period of conditioning: Six hours after the administration of morphine, at 18 h, animals were back in the home-cage, and the last temperature of the day was taken. An analysis of variance done on the reading for Days 6 to 9 revealed no significant effects (Appendix A, Table 16). The analysis done on the readings every seven days over the period of morphine administration yielded both a significant Group effect and a significant Group x Day interaction, but no Day effect (Appendix A, Table 17). The mean body temperature of animals in all groups averaged over Days 7, 14, and 21 and Days 70, 77, and 84 are shown in Figure 3. It would appear that towards the end of the experiment, when Group 4 was receiving extremely high doses of morphine, it was still hyperthermic at 18 h. A comparison of Days 7, 14, and 21 and Days 70, 77, and 84 in a Group x Block x Days analysis revealed a significant Block x Group interaction suggesting that there was a real difference between groups for these two periods (Appendix A, Table 18).

The Pre-Injection Conditioned Effect

As was mentioned in the beginning of the Results section, animals in the three morphine groups became hypothermic,

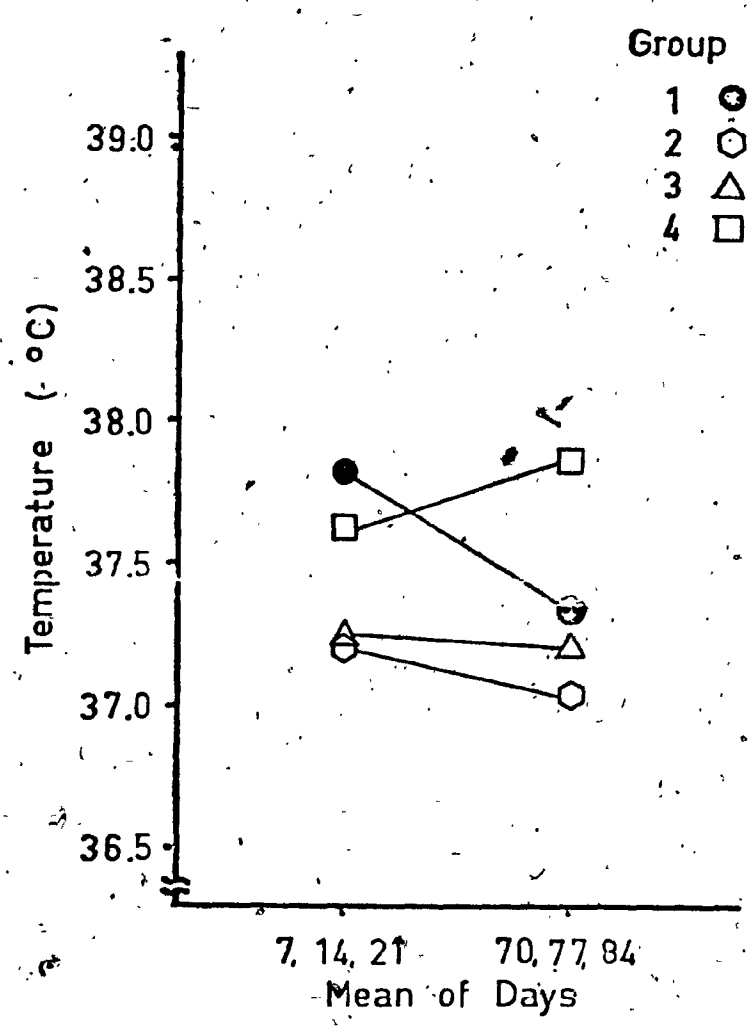

Figure 3: Mean body temperature of animals in the four groups at 18 h in the home-cage averaged over Days 7, 14, and 21 and over Days 70, 77, and 84.

relative to the animals receiving saline, in the pre-injection environment. It should be pointed out, however, that the pre-injection temperature measurement was taken not only one hour prior to the daily injection of morphine, but that it normally occurred, by necessity, 23 hours after the previous injection of morphine. It might be, therefore, that any difference observed between the morphine and the saline groups in the pre-injection environment were due to physiological symptoms of morphine deprivation, "physiological withdrawal", rather than to conditioned anticipatory responses to the oncoming injection. The analyses of the results, therefore, required the making of a complex set of comparisons in order to differentiate between these two interpretations. These will be presented in the following paragraphs.

Development of the pre-injection hypothermia: The group means for the pre-injection temperature readings taken on Days 6 to 9 are shown in Figure 4. These data were analysed using a Group x Days analysis of variance (Appendix A, Table 19). Both factors, and the interaction, were significant. As can be seen from Figure 4, the three morphine groups became increasingly hypothermic relative to the saline group over these four days. While the animals in the morphine groups did not differ from each other, there was a significant difference between animals in the three morphine groups and Group 1, the saline group, on Day 9, as revealed by a Scheffé test ($p < .05$). These data demonstrate that the pre-injection hypothermic effect developed rapidly, and at

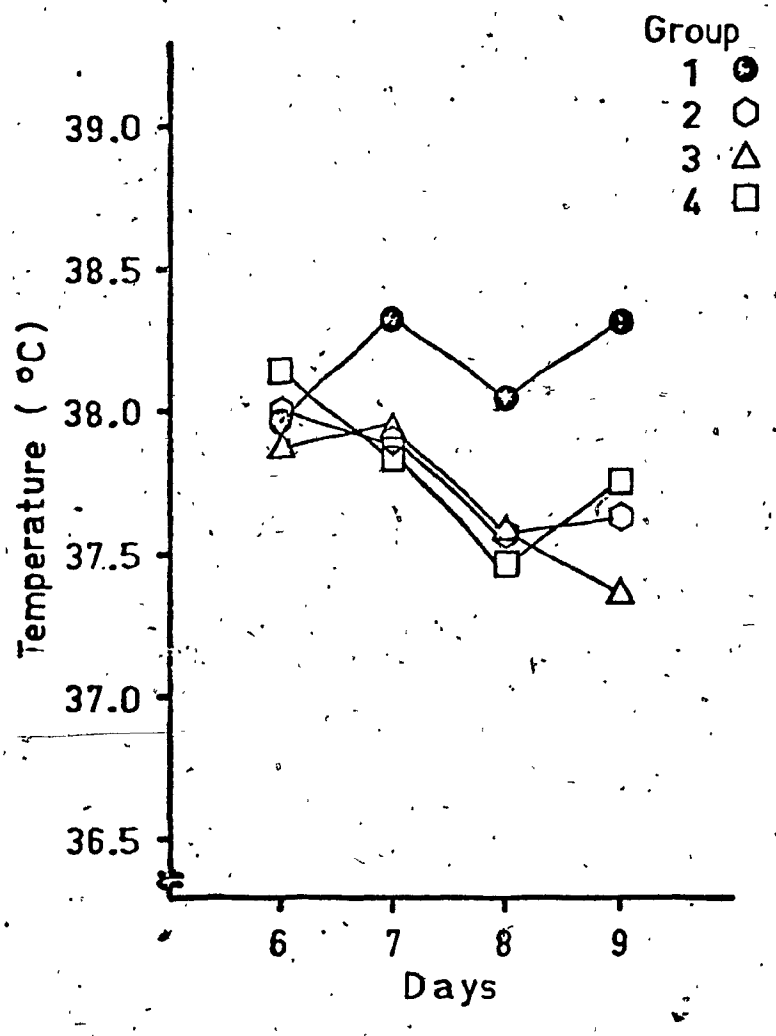

Figure 4: Mean body temperature of animals in the four groups at 11 h in the pre-injection environment for Days 6 to 9.

a similar rate, in all morphine groups regardless of dose.

This pre-injection effect was analysed further by following it over the course of the experiment. This was done by analysing the 11 h temperature readings for every seventh day, from Days 7 to 84, in a Group x Day analysis of variance (Appendix A, Table 20). As both main effects and the interaction were significant, the possibility of a trend over the experiment demanded an additional analysis. This was accomplished by comparing the first three days with the last three days of the above analysis, in a Group x Block x Day design (Appendix A, Table 21). All main effects, the Group x Block and Group x Block x Day interactions were all significant. The mean group temperatures for each Block is plotted in Figure 5. It can be seen that animals receiving the small dose of morphine (Group 2) became less hypothermic relative to the saline group (Group 1) over the course of the experiment, whereas the opposite was true of the other morphine groups (Groups 3 and 4). The significant Group x Block x Day interaction was probably due to the fact that, on Day 7, the hypothermic effect had not completely developed (see Figure 4). These analyses suggest that over the course of the experiment the strength of the effect became dose related, gradually attenuating in anirals receiving the small dose of morphine (Group 2).

Conditioning versus withdrawal:

Before attempting to differentiate between the role of "physiological withdrawal" and conditioning factors in the

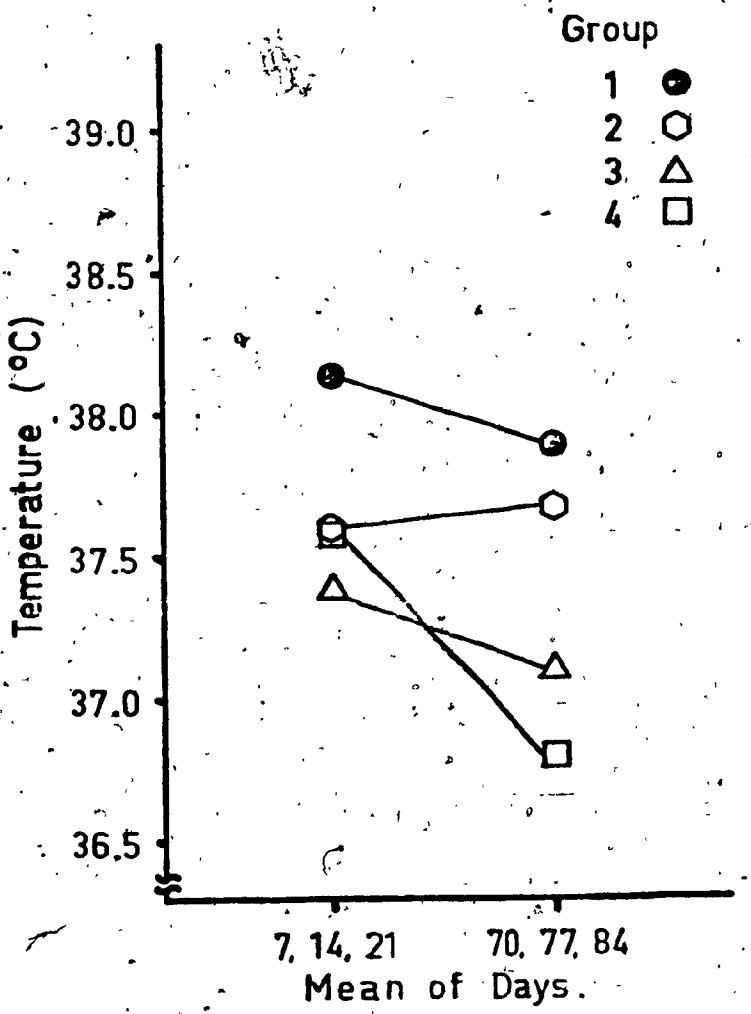

Figure 5: Mean body temperature of animals in the four groups at 11 h in the pre-injection environment averaged over Days 7, 14, and 21 and Days 70, 77, and 84.

production of the pre-injection hypothermia, evidence will be presented that at least some animals receiving morphine were dependent. It should be retalled that on the test days, Days 37, 43, 79, and 84, none of the animals were injected with morphine; as a result, on the days following these morphine-free days, at the time of the 9 h reading, all animals had been deprived of morphine for 45 hours. If any of the animals were physiologically dependent on morphine, one would have expected to observe weight loss over these two days. The average weight of animals in each of the groups on these eight days is shown in Figure 6. Data were analysed using a Group x Time x Replication design in which weights taken at 9 h on each pair of consecutive days, were considered as one replication (Appendix A, Table 22). All main effects and two-way Group interactions were significant. It is evident from Figure 6 that the animals receiving the larger doses of morphine (Groups 3 and 4) gained less weight over the course of the experiment than the saline group. In fact, animals in Group 4, that had been receiving the highest dose of morphine, showed a drop in weight of 20 to 30 g during each 48-hour period that they did not receive morphine. Thus these animals were clearly physically dependent by a widely accepted criterion.

Return now to the temperature differences in the pre-injection environment. If these differences had been due simply to the physiological effects of morphine deprivation, then, on those days when morphine was not administered, the temperature

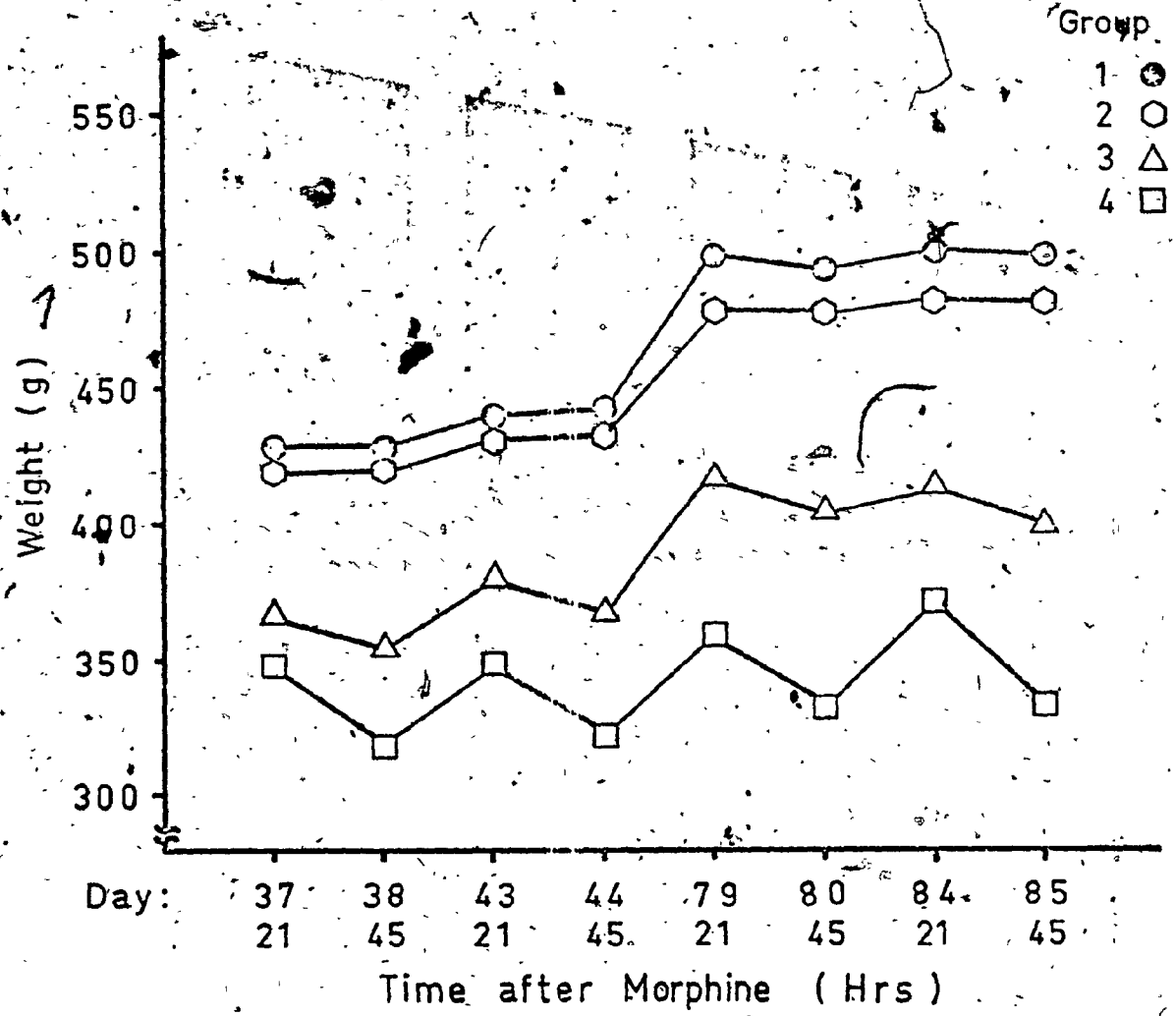

Figure 6: Mean weight of animals in the four groups for four pairs of consecutive days when morphine was not administered for 48 hours.

differences between the morphine and the saline groups should have increased over time, and then decreased as the animals recovered. Furthermore, these changes should have occurred regardless of the environment. If, however, the pre-injection differences between the groups had been due to conditioning factors, they should have been evident only in the presence of the conditioned stimuli, the pre-injection environment. There were two testing occasions when 48 hours intervened between morphine injections. On Days 37 and 38, and again on Days 79 and 80, body temperatures were measured in both the home-cage and the pre-injection environment over a 48-hour period. The 9 h and 11 h temperature readings taken on the two consecutive days represent readings taken 21, 23, 45, and 47 hours after the last morphine administration. The result, for the individual times over the two occasions, were collapsed and are presented in Figure 7. The results were analysed separately for each time using Group x Replication analysis of variance (Appendix A, Tables 23 to 26). It can be seen from Figure 7 that when animals were in the pre-injection environment, 23 and 47 hours after morphine, there was a pronounced difference between the groups; animals in the morphine groups had lower body temperatures than those in the saline group. The analyses of variance for both these times revealed similar results, significant Group and Replication effects but no significant interaction (Appendix A, Tables 23 and 24). Thus despite the overall difference between the two replications, the group difference for these two times after

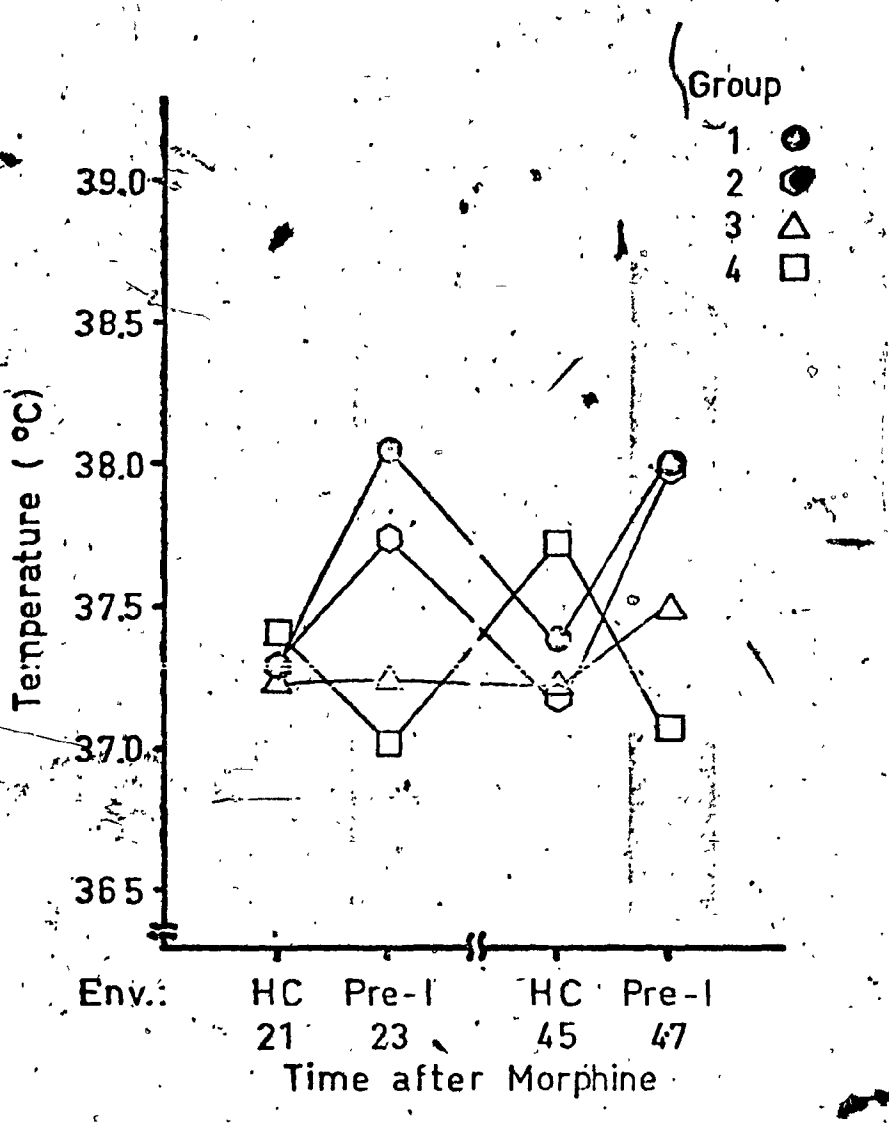

Figure 7: Mean body temperature of animals in all groups at 9 h in the home-cage (HC) and at 11 h in the pre-injection environment (Pre-1) over the time morphine was not administered for 48 hours.

morphine were consistent on the two occasions. Further, Figure 7 shows that in the home-cage, at 21 and 45 hours after morphine administration, the difference between the groups was much reduced and the ordering of the group means is clearly different from that at 23 and 47 hours after morphine. The Group x Replication analysis of variance revealed that at 21 hours after morphine only the Replication effect was significant, indicating that at 9 h there were no temperature differences between the groups (Appendix A, Table 25). Forty-five hours after morphine administration the analysis of variance revealed that the Group effect, Replication effect, and the interaction were significant (Appendix A, Table 26). However, examination of the means for Group 1 and Group 4, the saline and the high dose of morphine group, revealed that for both replications the Group 4 temperatures were higher than those in Group 1. Furthermore, it can be seen from Figure 7 that the temperature of animals in Group 4 was always lower in the pre-injection environment than it was in the home-cage. Thus, the hypothermic effect is evident on both occasions in the pre-injection environment, but not at the intervening time in the home-cage. This suggests that the pre-injection hypothermic effect is a conditioned anticipatory response.

Environmental specificity and the pre-injection hypothermia: The degree to which the pre-injection hypothermia (the 11 h temperature) was specific to the pre-injection environment was assessed by comparing the 11 h temperature readings taken

in the pre-injection environment, Days 37, 51, 65, and 79, to the 11 h reading taken in the home-cage, Days 43, 57, 71, and 85. The mean group temperature for each set of days is shown in Figure 8. Data were analysed by a Group x Environment x Replication analysis of variance (Appendix A, Table 27). All main effects and all two-way interactions were significant. Because the test trials were carried out at widely separated times over the period of morphine administration, and because the pre-injection effect changed over time as mentioned previously (see Figure 5), it is not surprising that the Replication effects and the Group x Replication, and Environment x Replication interactions proved to be significant (Appendix A, Table 27). It is the Group effect and Group x Environment interaction, however, that are of primary interest. As can be seen from Figure 8, the group differences are greater in the pre-injection environment than in the home-cage. The fact that the Group x Environment interaction was significant indicates that the pre-injection hypothermia was differentially affected by the environment, and suggests that pre-injection environmental cues were acting as conditioned stimuli for eliciting the effect. It should be noted, however, that there were some, though considerably smaller, differences between groups even in the home-cage environment, suggesting that temporal cues were also acting as conditioned stimuli for the hypothermic effect.

Termperature during abstinence: On Day 84 of the experiment, animals received their last morphine injection. The

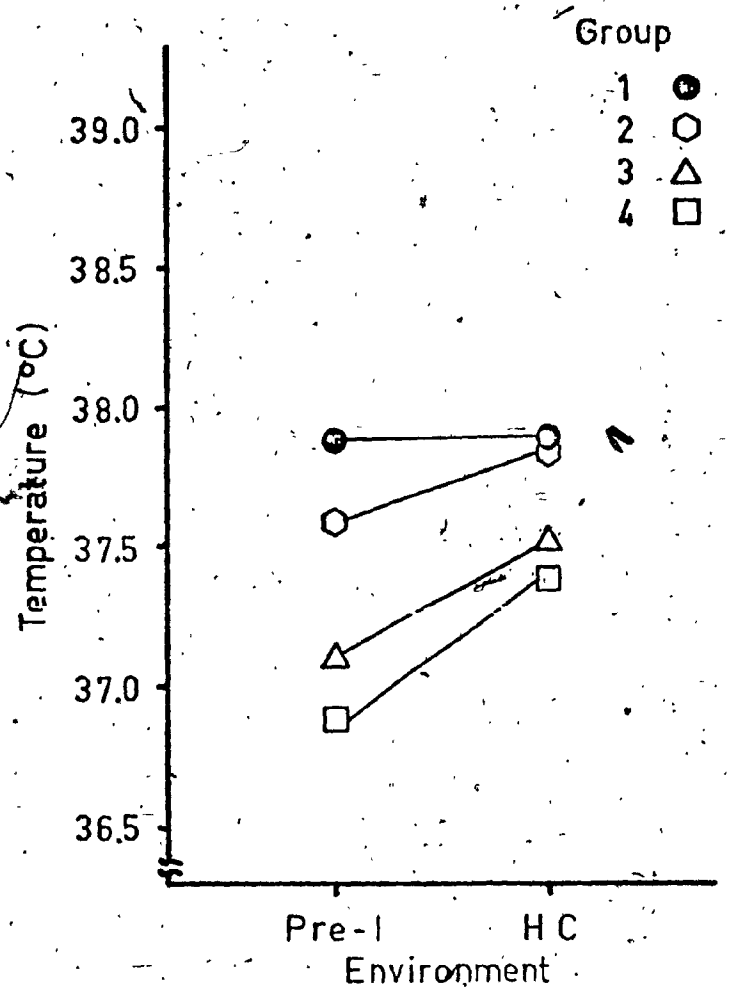

Figure 8: Mean body temperature of animals in all groups at 11 h averaged over the saline test days in the pre-injection environment (Pre-1) and those in the home-cage (HC).

next ten days, Days 85 to 94, were spent in the home-cage. The mean temperature readings for the four groups taken at 9 h are shown in Figure 9. It is apparent that at 9 h there was no consistent difference between the groups. A Group x Day analysis of variance carried out on the data for the first five days (Days 85 to 89) yielded only a significant Day effect, confirming the lack of a group difference (Appendix A, Table 28).

Figure 10 presents the data from the 11 h readings over the same period. As can be seen, there was an initial, dose-related, difference between the groups that disappeared over days. The Group x Days analysis for the first five days yielded significant Group and Days effects, but no significant interaction (Appendix A, Table 29). A further analysis of the 11 h temperature reading was done to compare Days 85, 86, and 87 to Days 92, 93, and 94. For this a Group x Block x Days analysis of variance was used (Appendix A, Table 30). The only significant effect was the Group x Block interaction. It is evident from Figure 10 that this reflects the fact that, at the beginning of the abstinence period there were group differences, but by the end of the abstinence period the difference at 11 h had disappeared. Note further that the larger the dose of morphine administered the more slowly the effect disappeared.

After abstinence: After the period of abstinence, on Day 95, and again on Day 121, the animals were returned to the pre-injection environment and temperatures were taken at

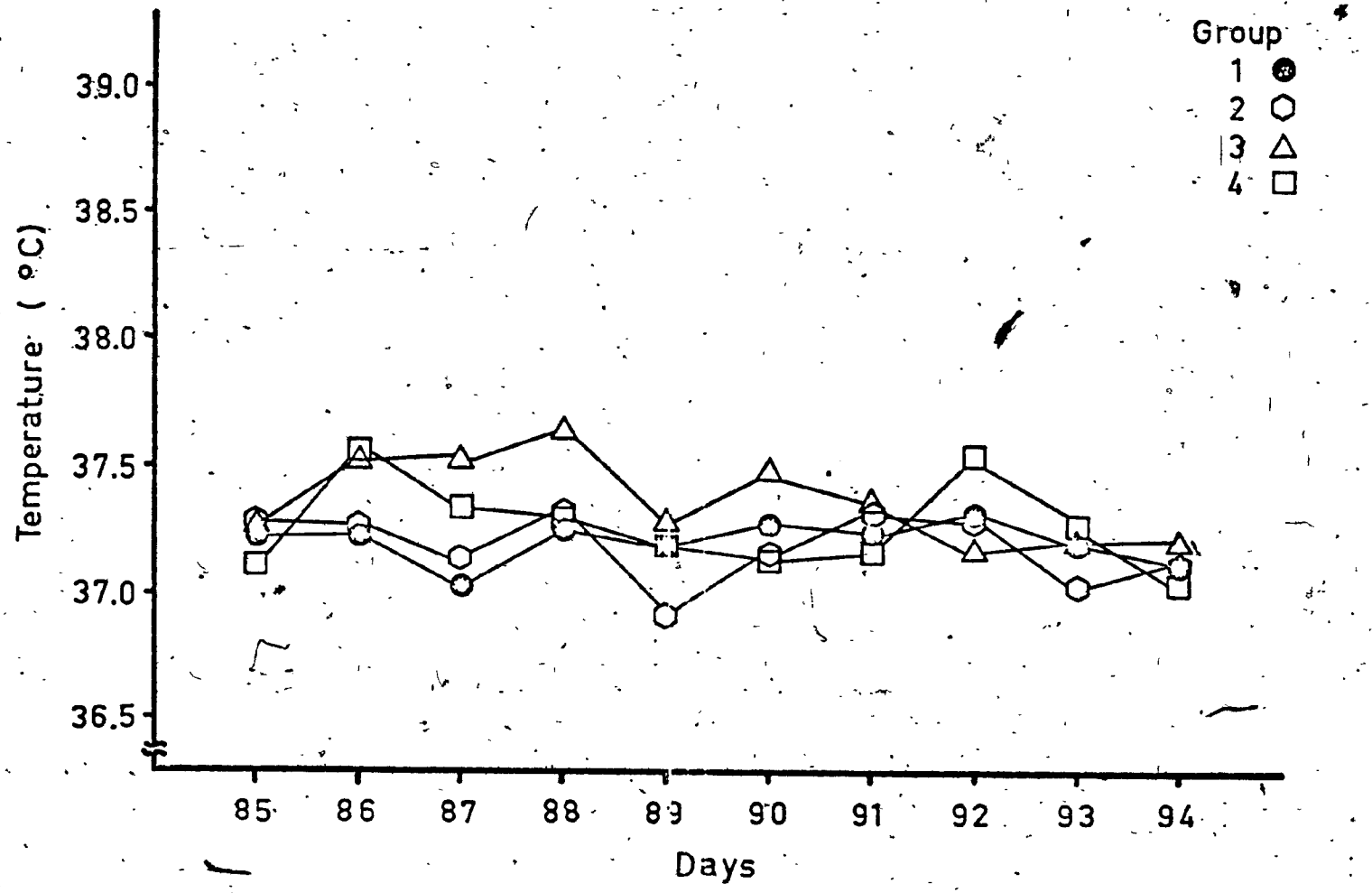

Figure 9: Mean body temperature of animals in all groups at 9 h in the home-cage for the days of morphine abstinence.

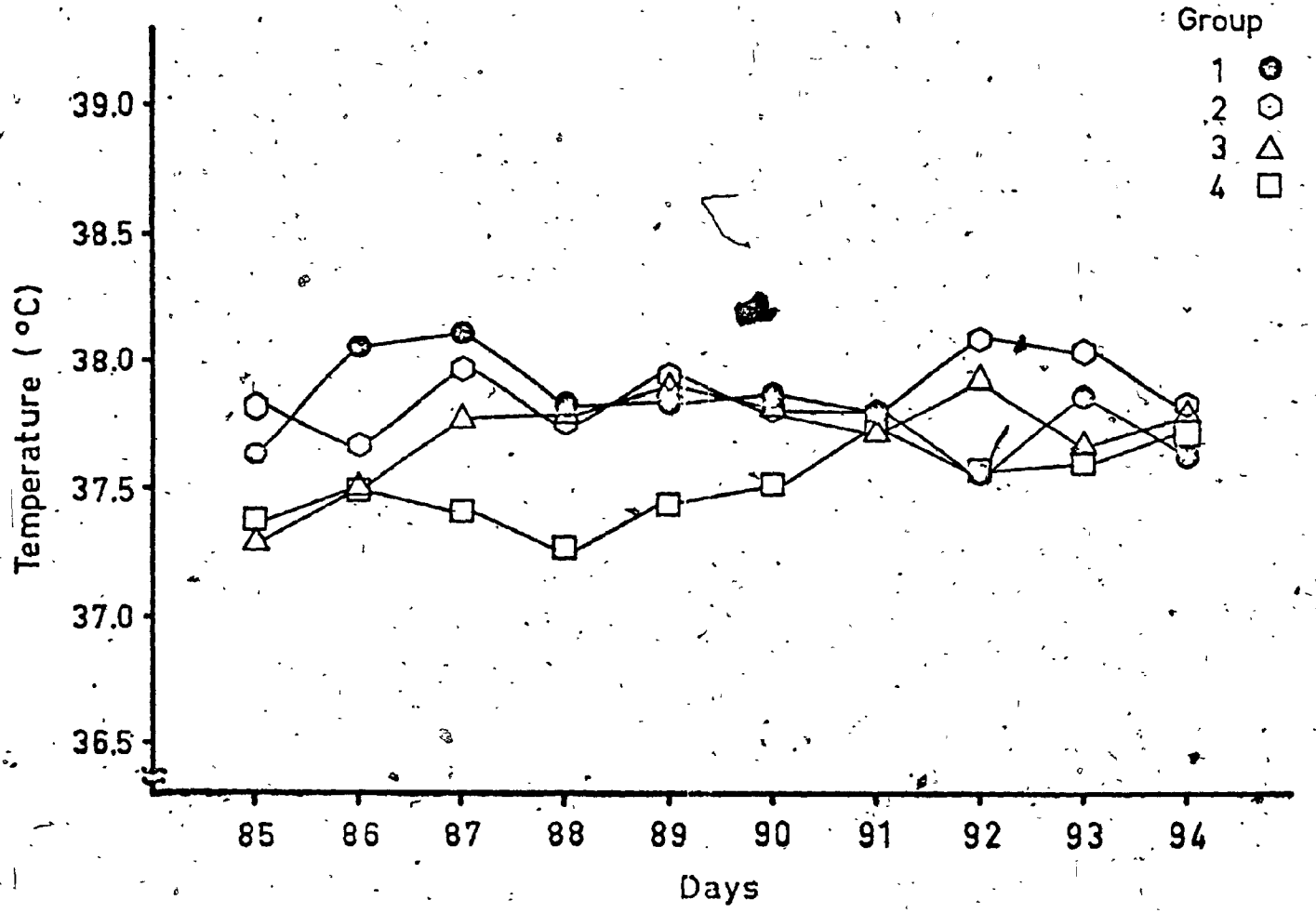

Figure 10: Mean body temperature of animals in all groups at 11 h in the home-cage for the period of abstinence.

11 h. In order to test for conditioned temperature responses to the pre-injection environmental stimuli a comparison was made between the temperature readings on Days 95 and 121 and those on Days 94 and 120 (days on which the animals remained in the home-cage). A comparison of the 9 h temperature readings on these days using a Group x Environment x Replication analysis of variance revealed that neither the Group effect nor any interaction involving Groups reached significance (Appendix A, Table 31). A similar Group x Environment x Replication analysis of variance for the 11 h temperature revealed similar findings (Appendix A, Table 32). Thus after ten days of abstinence the pre-injection hypothermia effect was no longer apparent in the pre-injection environment. To provide further confirmation for this finding, the 11 h temperature readings for the first four days in the pre-injection environment, Days 95 to 98, were analysed using a Group x Day analysis of variance (Appendix A, Table 33). Only the Day effect was significant and neither the Group effect nor the Group x Day interaction were significant.

The Post-Injection Conditioned Effect

In the case of the pre-injection data, because there was no significant difference in temperature of the four groups of animals at 9 h, it was possible to make comparisons between the groups at 11 h and to assume that any differences were due to the experimental manipulations. Any differences, however,

found at a later time (12:45 h and 14:15 h in the post-injection environment) could have been due either to the conditioned pre-injection hypothermia evident at 11 h or to intervening experimental manipulations. This made comparisons between the groups in the post-injection environment, during the period of morphine administration, difficult to interpret. The same problem, however, did not exist in the period after abstinence. As was seen above, after abstinence there were no longer any group differences in temperature at 11 h in either the home-cage or the pre-injection environment. For this reason, temperature differences between the groups in the post-injection environment in the period after abstinence will be considered first.

Tests for conditioning after abstinence: The mean temperature readings taken at 14:15 h for the four groups on Days 93 to 122 are shown in Figure 11. It is evident that at 14:15, animals in the morphine groups were hyperthermic in the post-injection environment, not only compared to animals in the saline group (Group 1), but also compared to their own temperatures taken at the same time in the home-cage on other days. In order to test for the situation-specificity of the post-injection hyperthermia, the temperature measurements taken at 12:45 h and at 14:15 h were analysed using separate Group x Environment x Replication analysis of variance, comparing the home-cage Days 94 and 120, to the post-injection environment Days 95 and 121 (Appendix A, Tables 34 and 35). At 12:45 h the Environment and the Replication effects, and

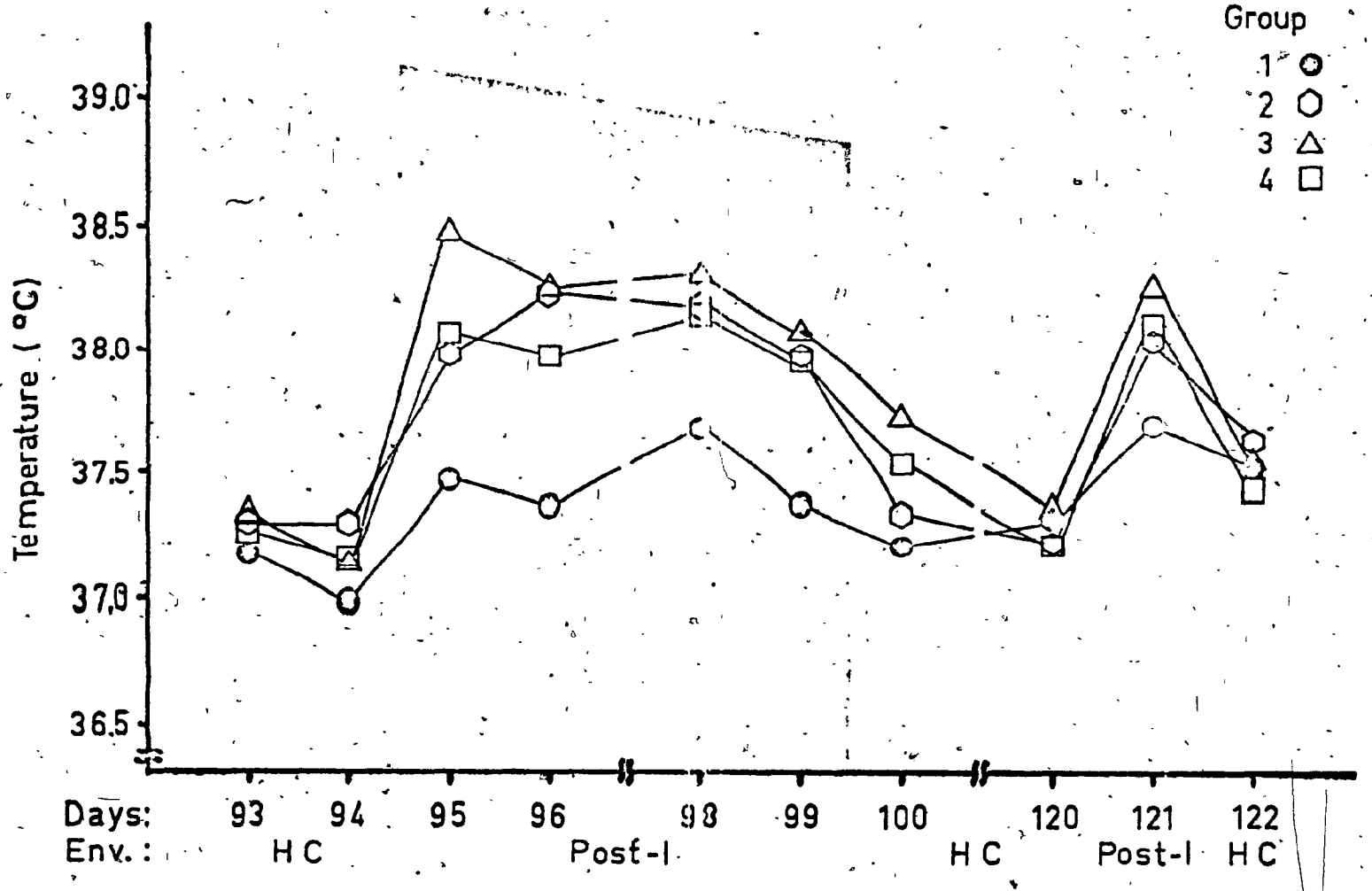

Figure 11: Mean body temperature of animals in the four groups at 14:15 h in the period after abstinence in either the home-cage (HC) or the post-injection environment (Post-1).

the Environment x Replication interaction were significant suggesting that the mean temperature for each of the four days included in this analysis was different. However, neither the Group or Group x Environment effects were significant suggesting that at 12:45 no real group differences had emerged. In contrast the analysis of the 14:15 h temperature measurements revealed that only the Environment effect and the Group x Environment interaction were significant (Appendix A, Table 35). As can be seen from Figure 11, these significant effects reflect a greater difference between the groups in the post-injection environment than in the home-cage.

Test for conditioning during the period of morphine administration: There were two sets of saline test days during the period of morphine administration. Each set consisted of a day in the post-injection environment (Days 37 and 79), paired with a day in the home-cage (Days 43 and 85). The mean group temperatures for these four days at 12:45 h and at 14:15 h are shown in Figures 12 and 13 respectively. Again separate Group x Environment x Replication analyses of variance were done for the 12:45 h and 14:15 h temperatures (Appendix A, Tables 36 and 37). At 12:45 h the only significant effects were the Group x Environment interaction and the Replication effect (Appendix A, Table 36). From Figure 12 it can be seen that in the post-injection environment animals in the morphine groups had lower body temperatures than animals in the saline group (Group 1), but that in the home-cage no such differences existed. At 14:15 h the three main effects

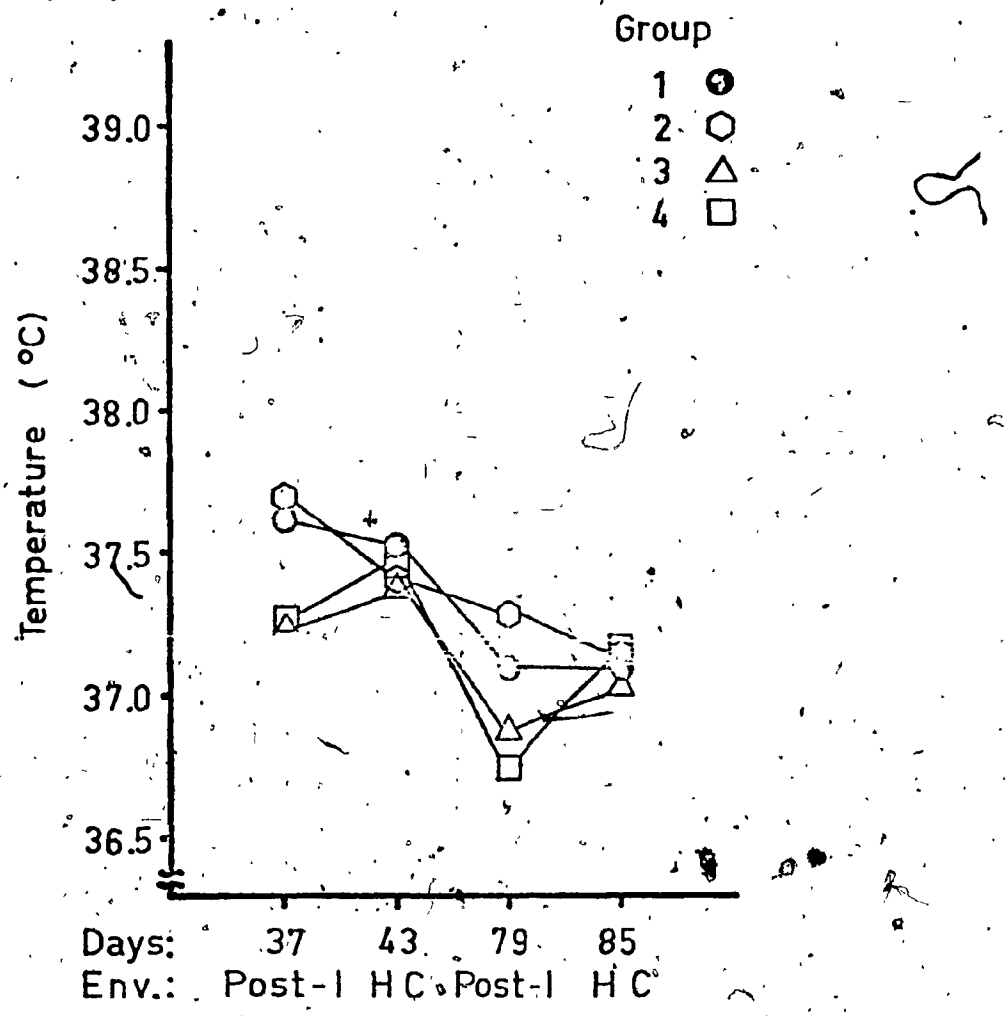

Figure 12: Mean body temperature of animals in the four groups on saline test days at 12:45 h in either the post-injection environment (Post-I) or the home-cage (HC).

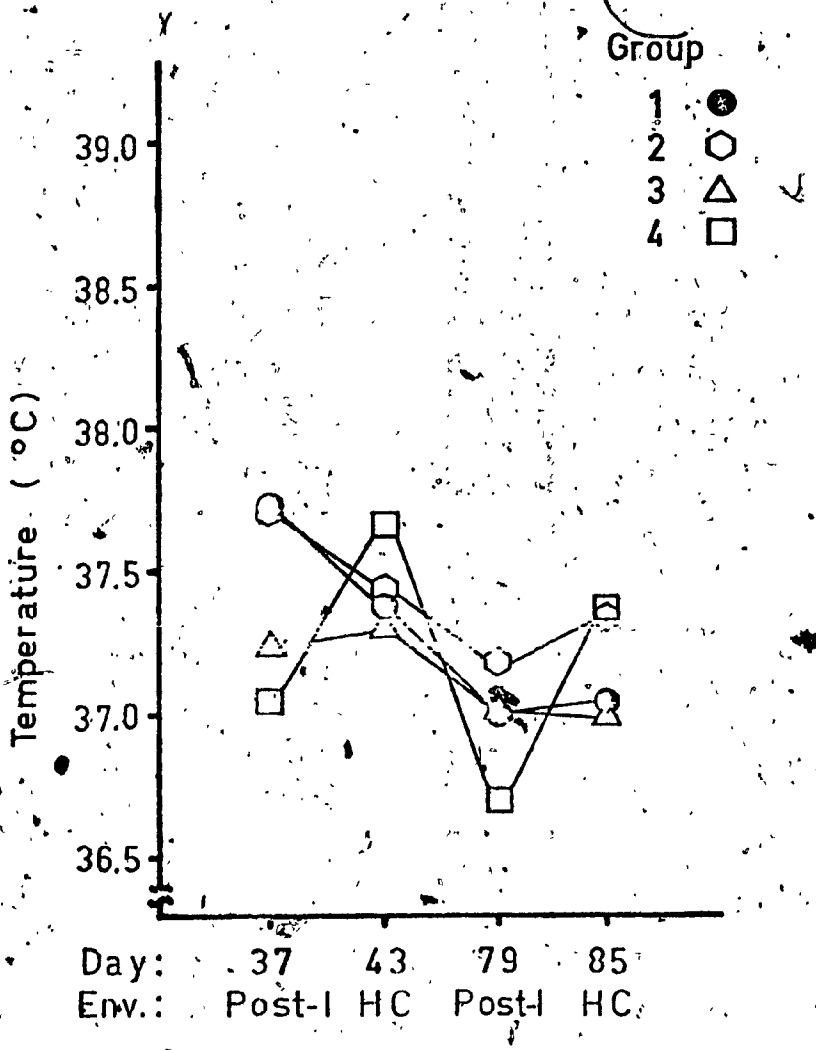

Figure 13: Mean body temperature of animals in the four groups on saline test days at 14:15 h in either the post-injection environment (Post-I) or the home-cage (HC).

and the Group x Environment interactions were all significant (Appendix A, Table 37). Although there appeared to be group differences in both environments only in the post-injection environment were these differences dose related. Note in Figure 13 that Group 4, the high dose of morphine group, was hypothermic relative to the saline group (Group 1) in the post-injection environment but hyperthermic relative to Group 1 in the home-cage.

Difference measures of the post-morphine effect: The difference between the post-injection temperature effect measured during the period of morphine administration and that measured after abstinence could have been due to the difference between the groups at 11 h during these two periods. In order to compare the results from the two periods more directly, the difference in temperature between 11 h and 12:45 h or between 11 h and 14:15 h was calculated for each animal. If the difference scores found in the period of morphine administration were similar to the difference scores found in the period after abstinence, it would suggest that the post-injection hyperthermia found in the period after abstinence (see Figure 11) was masked during the period of morphine administration by the pre-injection hypothermic response. Figure 14 shows the mean difference scores between 11 h and 12:45 h and between 11 h and 14:15 h on test days during morphine administration, Days 37 and 79, and after abstinence, Days 95 and 121. It is evident that scores in the two periods are very similar. Separate Group x Period x Replication analyses of

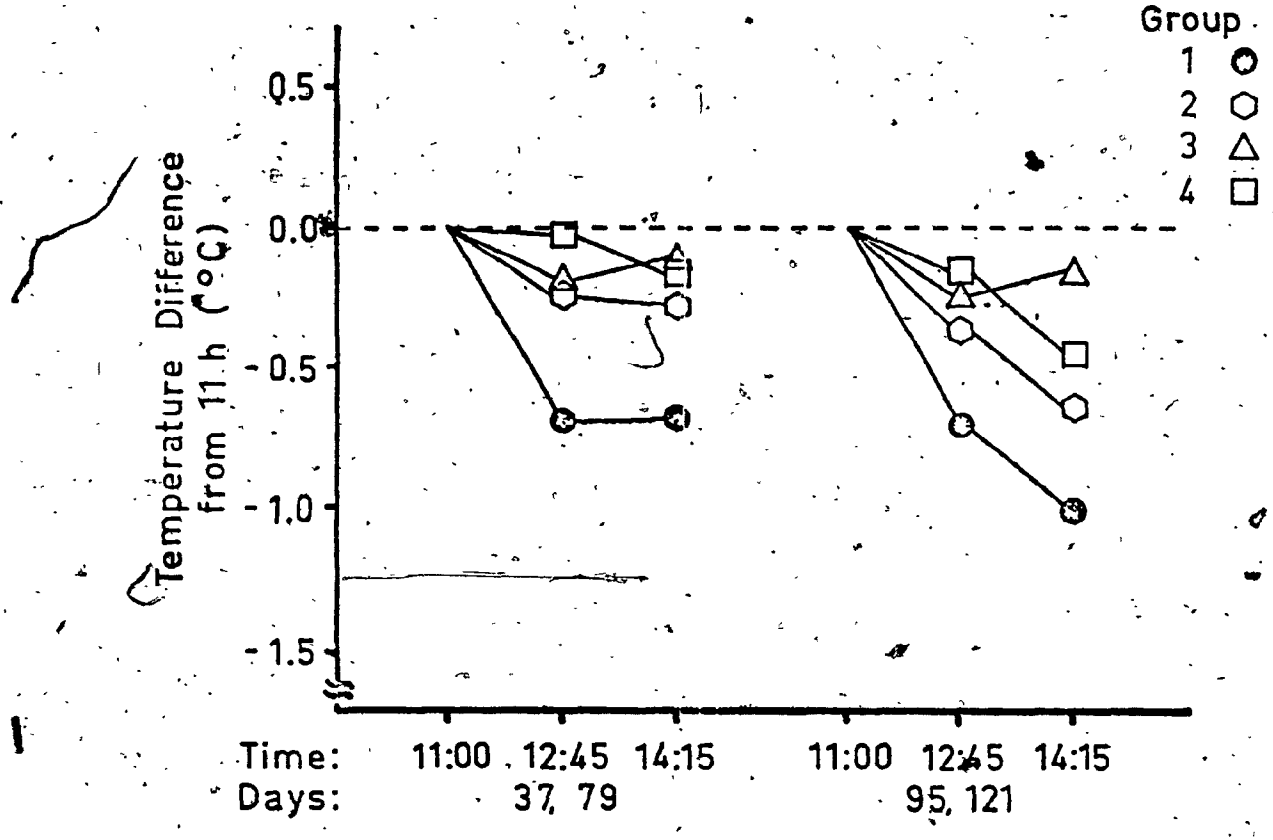

Figure 14: Mean difference in body temperature from 11 h of animals in the four groups at 12:45 h and 14:15 h in the post-injection environment on saline test days during the period of morphine administration (Days 37 and 79) and after abstinence (Days 95 and 121).

variance were done for the 12:45 h and the 14:15 h data (Appendix A, Tables 38 and 39). The analysis of the difference scores between 11 h and 12:45 h revealed only a significant Group effect. This suggests that the group-dependent changes in body temperature were virtually the same in the two periods. A very similar pattern of results was obtained for the difference scores between 11 h and 14:15 h (see Figure 14). The analysis of variance, however, revealed that there were significant Group, and Period effects and a significant Group x Period x Replication interaction. The significance of the interaction can be seen from Figure 15 in which the difference scores for the four test days are plotted. It is evident that the differences between the groups were getting larger during the period of morphine administration (the acquisition phase of conditioning) and smaller over trials in the period after abstinence (the "extinction" phase). This suggests that it was the difference between acquisition and "extinction" that resulted in the three-way interaction.

Tests using naloxone and 5 mg/kg of morphine

Naloxone and the low dose of morphine were given instead of the usual morphine injections on different test days during the period of morphine administration in order to determine how their effects might interact with any conditioned effects evident in the post-injection environment. However, the post-injection conditioned effect in itself was difficult to analyse

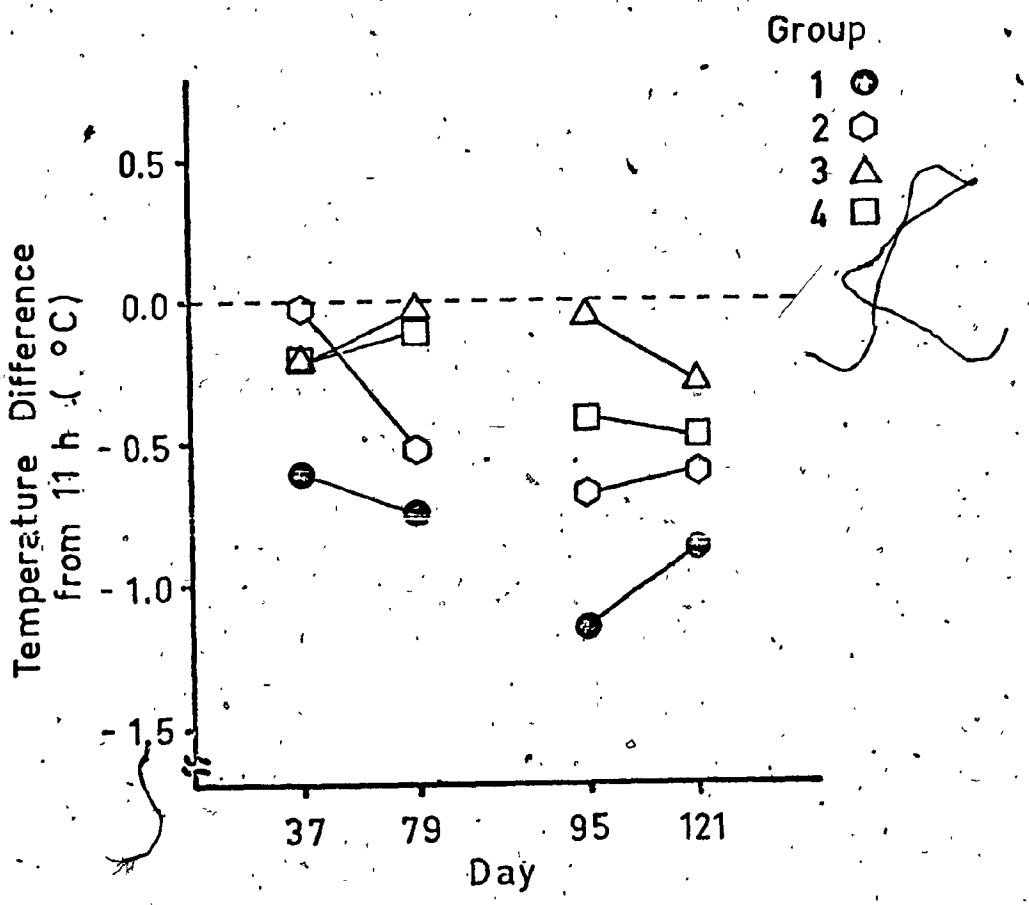

Figure 15: Mean difference in body temperature from 11 h of animals in the four groups at 14:15 h in the post-injection environment on saline test days during the period of morphine administration (Days 37 and 79) and after abstinence (Days 95 and 121).

because of the large differences in the 11 h pre-injection temperature readings. For this reason the effects of the naloxone and the 5 mg/kg morphine test were not analysed and will not be reported. It was possible, however, to consider the effect of naloxone given at 12 h in the period after abstinence when there was no longer any difference in 11 h temperatures between groups. The 12:45 h and the 14:15 h data were analysed by doing separate Group x Day analyses of variance for each time, comparing a saline test day, Day 96, with a naloxone test day, Day 97 (Appendix A, Tables 40 and 41). The results were identical for each time, there were significant Group and Day effects but no interaction. This suggests that while naloxone may have had a general effect on temperature (the Day effect) it did not disrupt the group differences. The morphine groups were hyperthermic relative to the saline group and maintained their dose-related positions after both a saline and a naloxone injection.

Discussion

In the present study two different conditioned temperature responses to morphine are evident. One, the conditioned hypothermia developed rapidly, was elicited in the pre-injection environment before the injection of morphine, and disappeared after a period of abstinence from morphine. The other, the conditioned hyperthermia, was evident in the post-injection environment and manifested itself most clearly in the period after abstinence from morphine. These results would seem to bear directly on the contradictory findings reported in previous studies of conditioned temperature responses to morphine. While Miksic et al (1975) and Roffman et al (1973) have reported a conditioned hyperthermic response, Siegel (1976, Note 1) has evidence that the conditioned response to morphine is a hypothermia. It now seems apparent that these differences may not only arise from methodological factors such as the different control temperature readings used in these studies, as was suggested in the introduction, but also from the fact that in these studies the immediate pre-injection environment was the same as the post-injection environment.

Before discussing in detail how the present results can be compared to those found in the previous studies, mention must be made of an unfortunate feature of the baseline temperature readings in the present experiment. It will be recalled that there were differences in body temperature at different times during the

day in the saline baseline period (see Figure 1) and that these temperature differences were evident in animals in the saline control group throughout the study. The reason for these temperature differences is not clear, but they do seem to be under control of temporal factors. During baseline days animals were warmest at 11 h in the pre-injection environment. This "rise" in temperature at 11 h was evident in the saline group throughout the study both on days the animals were in the pre-injection environment and on days when they remained in the home-cage (see Figure 8). Because of these differences, comparisons between the absolute temperature of morphine animals at one time and their temperature at another time were difficult to interpret. The appropriate temperature comparisons, therefore became those between the groups of morphine-treated animals and those of the saline group.

Recall that Siegel (1976, Note 1) reported that the conditioned response to the stimuli associated with repeated administration of small doses of morphine was hypothermia when comparisons were made between the morphine treated animals and a saline control group. In the present study, a similar conditioned effect was noted in the pre-injection environment prior to the morphine administration. Furthermore, during the period of morphine administration, the conditioned response evident in the post-injection environment was still a hypothermia relative to the saline control group. These findings, considered as such, confirm Siegel's and supports the view that the conditioned response to morphine is an

anticipatory hypothermic response opposite in direction to the unconditioned response to morphine.

The finding of an anticipatory hypothermic response is of interest for another reason, and bears on the usual interpretation of "withdrawal symptoms". The doses of morphine used in the present study range from small to large. Because the large dose produced dependence as measured by weight loss, the possibility that the pre-injection hypothermia was a physiological response to the temporary absence of morphine from the body had to be considered. This was especially important to consider since, over the course of the experiment, the magnitude of the effect proved to be dose-related (see Figure 5). It was demonstrated, however, that the hypothermia could not be accounted for in purely physiological terms, but was a conditioned anticipatory response evident only in the pre-injection environment at 11 h and not at 9 h in the home-cage. Similarly, during the period of abstinence the group difference was evident on successive days at 11 h, but was not present during the intervening, 9 h, temperature readings (see Figures 9 and 10). This suggests that at least some of what are generally labeled "withdrawal symptoms" are really conditioned anticipatory responses to the oncoming morphine injection. It may be, therefore, that some symptoms, normally considered to represent pure physiological effects, are in fact conditioned effects inasmuch as as they are observed in the situation in which the animal normally receives the drug.

This interpretation can also be applied to findings from studies of the conditioning of withdrawal symptoms (Trost, 1973; Wikler & Pescor, 1967). As mentioned in the introduction, in these studies, the conditioned stimuli for withdrawal are usually presented and then terminated close to the time when the animals would be injected with their maintenance dose of morphine. Similarly, in the O'Brien et al (1977) study, the dose of naloxone was chosen so that its effects were short lasting, and termination of the conditioned stimuli coincided with reinstatement of the methadone effect. Thus, rather than conditioning withdrawal symptoms, it may be that in these studies an anticipatory response to the next injection of morphine, similar to that found in the present study, may be being conditioned.

One final feature of the conditioned anticipatory hypothermia found in the present study requires comment. Recall that the effect developed rapidly (see Figure 4) and was clearly evident throughout the period (see Figure 5) of morphine administration. The comparison of the 11 h temperature in the pre-injection environment and in the home-cage showed that both situational and temporal cues were important in eliciting the effect (Figure 8). At 11 h during the period of abstinence the hypothermic effect was evident for several days in the home-cage and then disappeared in a dose related fashion (see Figure 10). Surprisingly, when the animals were returned to the conditioning environment after the period of abstinence, there were no group differences in body

temperatures at 11 h. It was unexpected that stimuli that had previously been able to elicit the effect (see Figure 8) no longer did so, especially since there was no opportunity for extinction to occur to these environment cues.

There are two possible explanations for this loss of the conditioned anticipatory response over the period of abstinence. One is that temporal cues were the major or most salient conditioned stimuli for the conditioned anticipatory response. Morphine was always administered at 12 h creating the possibility that temporal cues were at least part of a complex of conditioned stimuli. There are two findings that appear to support this line of reasoning. While it is true that at 11 h the temperature differences between the animals in the morphine and saline groups were larger in the pre-injection environment, there were differences between the groups on days when the animals remained in the home-cage (see Figure 8). Further, when animals remained in the home-cage, during the period of abstinence, the difference between the morphine and saline groups was evident at first, but disappeared over several days, in a dose related fashion. It may have been that because morphine was no longer administered at 12 h during the period of abstinence, that the response normally elicited by the temporal cues extinguished. If this were the case, it is possible that on return to the pre-injection environment, because the response to the temporal cues had been extinguished the effect could no longer be elicited even by the complete complex of

conditioned stimuli.

An alternative explanation is that the conditioned hypothermia was tied to an artificial deprivation state induced by the daily administration of morphine, much in the way that conditioned salivation would be dependent on food deprivation. It is possible that the hypothermic response, which was clearly time and situation dependent, required the deprivation state for its elicitation. Thus after the period of abstinence, when animals were no longer in the artificial deprivation state, the conditioned hypothermic response may no longer have been elicitable by the conditioned stimuli. The adequacy of either of these two possible explanations could be tested for in two ways. One would be to reinduce the morphine deprivation state by giving a few daily injections of morphine and then retesting for the conditioned response in the pre-injection environment. Alternatively, morphine could be administered to animals in a distinctive environment, but at random intervals, in order to prevent temporal cues from becoming part of any conditioned stimulus complex predictive of morphine. Under these circumstances extinction of the responses to the conditioned stimulus should not occur during the period of abstinence. If, after abstinence, the conditioned effect could still not be elicited by the cues of the distinctive environment, it would have to be concluded that the conditioned response was dependent on the presence of a state of artificial deprivation induced by morphine.

Turn now to the relevance of the present study, to

the findings of Miksic et al (1975) and Roffman et al (1973). It will be remembered that in their studies the conditioned response to morphine was reported to be hyperthermia or a mimicking of the unconditioned response to morphine. Miksic et al (1975) found that animals that had been receiving a 20 mg/kg dose of morphine paired with a tone, showed a rise in body temperature after presentation of the tone alone compared to their pre-tone temperature. Roffman et al (1973) studied conditioning in animals made dependent by repeated administration of high doses of morphine in the presence of a conditioned stimulus. They found that animals exposed to repeated presentations of the conditioned stimulus alone during a period of withdrawal from morphine, maintained higher body temperatures than animals not exposed to the conditioned stimulus. It would appear that in both of these studies, control temperature readings were taken in the same environment as were the post-conditioned stimulus temperature readings.

The comparable data from the present experiment are the post-injection temperature readings. A similar post-injection hyperthermia was apparent, but only clearly so, in the period after abstinence. During the period of morphine administration this conditioned effect was not evident; in fact, the morphine groups were hypothermic relative to the saline group in the post-injection environment. However, as mentioned in the results, this could have been due to the pre-injection hypothermia masking any post-injection effect. The situation is further complicated by the fact that

the saline control group body temperature was higher at 11 h than at any other time. The only way, then, in which to test for a similarity between the processes occurring during the period of morphine conditioning and those occurring after abstinence was to compare the changes in temperature that occurred between 11 h and the post-injection readings in the two periods. Given that the post-injection hyperthermia was a robust phenomenon in the period after abstinence, the analysis of the difference scores provide strong support that a similar process was occurring during the period of morphine administration.

Although it would be premature to speculate about its implications, the finding that naloxone did not block this post-injection, post abstinence hyperthermia, is intriguing and worthy of further study. This finding becomes especially interesting in light of reports that naloxone does have the ability to block the conditioning of hyperthermia in animals receiving daily doses of morphine (Drawbaugh & Lal, 1974; Lal et al, 1976).

The fact that the post-injection conditioned hyperthermia was clearly evident in the period after abstinence is both puzzling and facinating. Unlike the pre-injection conditioned anticipatory temperature effect, the post-injection conditioned hyperthermia did not disappear after the period of abstinence. Clearly this finding has important implications for questions concerning the role of conditioned factors in the resumption of morphine intake in individuals who have stopped taking morphine for

long periods of time. If long after a period of withdrawal from the drug, stimuli that have been associated with the effects of morphine continue to be able to elicit conditioned physiological changes, similar to those produced by morphine, our ideas about long term treatment programs will have to be altered.

In summary it can be said that the present study shows that the conditioned effects to morphine are much more complex and probably more important than previously suggested. Even within one response system, the temperature system, two different conditioned effects were apparent in the same animals, one opposing, and the other mimicking the unconditioned effects of morphine. Furthermore, the results suggest that anticipatory opponent conditioned responses may account for aspects of withdrawal symptoms whereas mimicking or "placebo" responses evident after a period of abstinence may be important in the resumption of drug use.

Reference Note

1. Siegel, S. Personal communication, Fall, 1976.

References

Ary, M., Chesarek, W., Sorensen, S. M., & Lomax, P. Naltrexone-induced hypothermia in the rat. European Journal of Pharmacology, 1976, 39, 215-220.

Babbini, M. & Davis, W. M. Time-dose relationship for locomotor activity effects of morphine after acute or repeated treatment. British Journal of Pharmacology, 1972, 46, 213-224.

Babbini, M., Gaiardi, M., & Bartoletti, M. Changes in fixed interval behavior during chronic morphine treatment and morphine abstinence in rats. Psychopharmacologia, 1976, 45, 225-259.

Bush, H. D., Bush, M. F., Miller, M. A., & Reid, L. D. Addictive agents and intracranial stimulation: Daily morphine and lateral hypothalanic self-stimulation. Physiological Psychology, 1976, 4, 79-85.

Collins, K. H. & Tatum, A. L. A conditioned salivary reflex established by chronic morphine poisoning. American Journal of Physiology, 1925, 74, 14-15.

Cox, B., Ary, M., Chesarek, W., & Lomax, P. Morphine hyperthermia in the rat: An action on the central thermostats. European Journal of Pharmacology, 1976, 36, 33-39.

Domino, E. F., Vasko, M. R., & Wilson, A. E. Mixed depressant and stimulant actions of morphine and their relationship to brain acetylcholine. Life Sciences, 1976, 18, 361-376.

Drawbaugh, R., & Lal, H. Reversal by narcotic antagonist of a narcotic action elicited by a conditioned stimulus. Nature, 1974, 247, 65-67.

Esposito, R., & Kornetsky, C. Morphine lowering of self-stimulation thresholds: Lack of tolerance with long-term administration. Science, 1977, 195, 189-191.

Glick, S. D. & Rapoport, G. Tolerance to the facilitatory effect of morphine on self-stimulation of the medial forebrain bundle in rats. Research Communications in Chemical Pathology and Pharmacology, 1974, 9, 647-652.

Goldberg, S. R., & Schuster, C. R. Conditioned suppression by a stimulus associated with nalorphine in morphine-dependent monkeys. Journal of Experimental Analysis of Behaviour, 1967, 10, 235-242.

Goldberg, S. R., Woods, J. H., & Schuster, C. R. Morphine: Conditioned increases in self-administration in Rhesus monkeys. Science, 1969, 166, 1306-1307.

Gunne, L. M. The temperature response in rats during acute and chronic morphine administration a study of morphine tolerance. Archives Internationale Pharmacodynamie et Therapie, 1960, 129, 416-428.

Herrmann, J. B. The pyretic action on rats of small doses of morphine. Journal of Pharmacology and Experimental Therapeutics, 1942, 76, 309-315.

Jaffe, J. H. Drug addiction and drug abuse. In L. S. Goodman & A. Gilman (Eds.), The pharmacological base of therapeutics (5th ed.). New York: Macmillan, 1975.

Jaffe, J. H., & Martin, W. R. Narcotic analgesics and antagonists. In L. S. Goodman & A. Gilman (Eds.), The pharmacological basis of therapeutics (5th ed.). New York: Macmillan, 1975.

Kamat, K. A., Dutta, S. N., & Pradhan, S. N. Conditioning of morphine induced enhancement of motor activity. Research Communications in Chemical Pathology and Pharmacology, 1974, 7, 367-373.

Kayan, S., Woods, L. A., & Mitchell, C. L. Morphine-induced hyperalgesia in rats tested on the hot plate. Journal of Pharmacology and Experimental Therapeutics, 1971, 177, 509-513.

Kleitman, N., & Crisler, G. A quantitative study of a salivary conditioned reflex. American Journal of Physiology, 1927, 79, 571-614.

Kumar, R. Morphine dependence in rats: Secondary reinforcement from environmental stimuli. Psychopharmacologia, 1972, 25, 332-338.

Lal, H., Miksic, S., & Smith, N. Naloxone antagonism of conditioned hyperthermia: An evidence for release of endogenous opioid. Life Sciences, 1976, 18, 971-976.

Lomax, P. Measurement of 'core' temperature in the rat. Nature, 1966, 210, 854-855.

Lomax, P., & Kirkpatrick, W. E. The effect of n-allylnormorphine on the development of acute tolerance to the analgesic and hypothermic effect of morphine in the rat. Medicina et Pharmacologia Experimentalis, 1967, 16, 165-170.

Lorens, S. A., & Mitchell, C. L. Influence of morphine on lateral hypothalamic self-stimulation in the rat. Psychopharmacologia, 1973, 32, 271-277.

Lotti, V. J., Lomax, P., & George, R. N-allynormorphine antagonism of the hypothermic effect of morphine in the rat following intracerebral and systemic administration. Journal of Pharmacology and Experimental Therapeutics, 1965, 150, 420-425.

Martin, W. R. Opioid antagonists. Pharmacological Review, 1967, 19, 463-521.

Martin, W. R., Wikler, A., Eades, C. G., & Pescor, F. T. Tolerance to and physical dependence on morphine in rats. Psychopharmacologia, 1963, 4, 247-260.

Miksic, S., Smith, N., Numan, R., & Lal, H. Acquisition and extinction of a conditioned hyperthermic response to a tone paired with morphine administration. Neuropsychobiology, 1975, 1, 277-283.

O'Brien, C. P., Testa, T., O'Brien, T. J., Brady, J. P. & Wells, B. Conditioned narcotic withdrawal in humans. Science, 1977, 195, 1000-1002.

Paolino, R. M., & Bernard, B. K. Environment temperature effects on the thermoregulatory response to systemic and hypothalamic administration of morphine. Life Sciences, 1968, 7(1), 857-863.

Pavlov, I. P. Conditioned reflexes (G. V, Anrep, trans.). London: Oxford University Press, 1927.

Roffman, M., Reddy, C., & Lal, H. Control of morphine-withdrawal hypothermia by conditional stimuli. Psychopharmacology, 1973, 29, 197-201.

Schuster, C. R. & Woods, J. H. The conditioned reinforcing effects of stimuli associated with morphine reinforcement. The International Journal of the Addictions, 1968, 3, 223-230.

Seevers, M. H., & Deneau, G. A. Physiological aspects of tolerance and physical dependence. In W. S. Root & F. G, Hofmann (Eds.), Physiological pharmacology (Vol. 1). New York: Academic Press, 1963.

Siegel, S. Evidence from rats that morphine tolerance is a learned response. Journal of Comparative and Physiological Psychology, 1975, 89, 498-506.

Siegel, S. Morphine analgesic tolerance: Its situation specificity supports a Pavlovian conditioning model. Science, 1976, 193, 323-325.

Siegel, S. Morphine tolerance acquisition as an associative process. Journal of Experimental Psychology: Animal Behavior Processes, 1977, 3, 1-13.

Trost, R. C. Differential classical conditioning of abstinence syndrome in morphine-dependent rats. Psychopharmacologia, 1973, 30, 153-161.

Tye, N. C., & Iversen, S. D. Some behavioural signs of morphine withdrawal blocked by conditioned stimuli. Nature, 1975, 225, 416-418.

Weinstein, S. H., Pfeffer, M., & Schor, J. M. Metabolism and pharmacokinetics of naloxone. In M. C. Brande, L. S. Harris, E. L. May, J. P. Smith, & J. E. Villarreal (Eds.), Narcotic antagonists: Advances in biochemical psychopharmacology (Vol. 8). New York: Raven Press, 1974.

Wikler, A., & Pescor, F. T. Classical conditioning of a morphine abstinence phenomenon, reinforcement of opioid-drinking behavior and 'relapse' in morphine-addicted rats. Psychopharmacologia, 1967, 10, 255-284.

Appendix A

Summary tables for the analysis of variance

TABLE 1

The analysis of variance of the effect of the time of day on the temperature of animals in all groups on days prior to the start of the experiment.

Source	df	MS	F
Group (G)	3	1.50	1.60
Subjects (S)	26	.94	
Time (T)	4	6.99	30.53**
G x T	12	.16	.72
T x S	104	.23	
Days (D)	3	.44	3.93*
G x D	9	.17	1.52
D x S	78	.11	
T x D	12	1.49	13.55**
G x T x D	36	.12	1.08
T x D x S	312	.11	

*$p < .05$.

**$p < .01$.

TABLE 2

The analysis of variance of the saline baseline temperature of animals in all groups for all times of Days 1 to 4.

Source	df	MS	F
Group (G)	3	.20	.18
Subject (S)	26	1.17	
Time (T)	4	12.59	46.81**
G x T	12	.70	2.60**
T x S	104	.27	
Days (D)	3	.30	2.01
G x D	9	.10	.63
D x S	78	.15	
T x D	12	2.98	16.83**
G x T x D	36	.15	.84
T x D x S	312	.18	

**p < .01.

TABLE 3

The analysis of variance of the saline baseline temperature of animals in all groups at 9 h in the home cage for Days 1 to 4.

Source	df	MS	F
Group (G)	3	.36	.61
Subject (S)	26	.59	
Days (D)	3	2.18	11.37**
G x D	9	.17	.88
D x S	78	.19	

**$p < .01$.

TABLE 4

The analysis of variance of the saline baseline temperature of animals in all groups at 11 h in the pre-injection environment for Days 1 to 4.

Source	df	MS	F
Group (G)	3	.47	1.78
Subject (S)	26	.27	
Days (D)	3	1.34	24.68**
G x D	9	.03	.62
D x S	78	.05	

**p < .01.

TABLE 5

The analysis of variance of the saline baseline temperature of animals in all groups at 12:45 h in the post-injection environment for Days 1 to 4.

Source	df	MS	F
Group (G)	3	.19	.59
Subject (S)	26	.32	
Days (D)	3	.27	5.22**
G x D	9	.02	.39
D x S	78	.05	

**p < .01.

TABLE 6

The analysis of variance of the saline baseline temperature of animals in all groups at 14:15 h in the post-injection environment for Days 1 to 4.

Source	df	MS	F
Group (G)	3	.27	.81
Subject (S)	26	.33	
Days (D)	3	.14	3.02*
G x D	9	.07	1.44
D x S	78	.05	

*$p < .05$.

TABLE 7

The analysis of variance of the saline baseline temperature of animals in all groups at 18 h in the home cage for Days 1 to 4.

Source	df	MS	F
Group (G)	3	.93	1.72
Subject (S)	26	.54	
Days (D)	3	7.30	48.45**
G x D	9	.12	.83
D x S	78	.15	

**$p < .01$.

TABLE 8

The analysis of variance of the temperature of animals in all groups at 9 h in the home cage on Days 6 to 9.

Source	df	MS	F
Group (G)	3	.27	.95
Subject (S)	26	.29	
Days (D)	3	.15	1.25
G x D	9	.09	.77
D x S	78	.12	

TABLE 9

The analysis of variance of the temperature of animals in all groups at 9 h, in the home cage for every seventh day of the experiment from Day 7 to Day 84 inclusive.

Source	df	MS	F
Group (G)	3	.69	2.07
Subject (S)	26	.33	
Days (D)	11	2.70	20.97**
G x D	33	.11	.83
D x S	286	.13	

**p < .01.

TABLE 10

The analysis of variance of the effect of an injection of morphine given at 12 h on the temperature of animals in all groups at 12:45 h for Days 6 to 9.

Source	df	MS	F
Group (G)	3	14.28	68.73**
Subject (S)	26	.21	
Days (D)	3	.31	1.86
G x D	9	.36	2.15*
D x S	78	.17	

*$p < .05$.

**$p < .01$.

TABLE 11

The analysis of variance of the effect of an injection of morphine given at 12 h on the temperature of animals in all groups at 14:15 h for Days 6 to 9.

Source	df	MS	F
Group (G)	3	38.56	98.91**
Subjects (S)	26	.39	
Days (D)	3	.06	.55
G x D	9	.06	.60
D x S	78	.10	

**p < .01.

TABLE 12

The analysis of variance of the effect of an injection of morphine given at 12 h on the temperature of animals in all groups at 12:45 h in the post-injection environment for every seventh day of the experiment from Day 7 to Day 84 inclusive.

Source	df	MS	F
Group (G)	3	72.87	95.61**
Subject (S)	26	.76	
Days (D)	11	1.28	9.29**
G x D	33	.31	2.24**
D x S	286	.14	

**$p < .01$.

TABLE 13

The analysis of variance of the effect of an injection of morphine given at 12 h on the temperature of animals in all groups at 14:15 h for every seventh day of the experiment from Day 7 to Day 84 inclusive.

Source	df	MS	F
Group (G)	3	105.28	261.15**
Subject (S)	264	.40	
Days (D)	11	1.05	9.13**
G x D	33	.20	1.71*
D x S	286	.12	

*p < .05.

**p < .01.

TABLE 14

The analysis of variance of the comparison of the effect of an injection of morphine given at 12 h on the temperature of animals in all groups at 12:45 h on Days 7, 14, and 21 and on Days 70, 97, and 84.

Source	df	MS	F
Group (G)	3	34.79	101.42**
Subjects (S)	26	.34	
Block (B)	1	.83	3.41
G x B	3	.68	2.79
B x S	26	.24	
Days (D)	2	1.82	15.08**
G x D	6	.12	.96
D x S	52	.12	
B x D	2	.71	5.18**
G x B x D	6	.18	1.33
B x D x S	52	.14	

**p < .01.

TABLE 15

The analysis of variance of the comparison of the effect of an injection of morphine given at 12 h on the temperature of animals in all groups at 14:15 h on Days 7, 14, and 21 and on Days 70, 77, and 84.

Source	df	MS	F
Group (G)	3	50.14	202.98**
Subject (S)	26	.25	
Block (B)	1	.50	3.06
G x B	3	.20	1.23
B x S	26	.16	
Days (D)	2	1.41	8.47**
G x D	6	.31	1.87
D x S	52	.17	
B x D	2	.43	2.93
G x B x D	6	.04	.26
B x D x S	52	.15	

**$p < .01$.

TABLE 16

The analysis of variance of the temperature of animals in all groups at 18 h (six hours after morphine administration) for Days 6 to 9.

Source	df	MS	F
Group (G)	3	.65	1.36
Subject (S)	26	.48	
Days (D)	3	.06	.26
G x D	9	.38	1.68
D x S	78	.22	

TABLE 17

The analysis of variance of the temperature of animals in all groups at 18 h (six hours after morphine administration) for every seventh day of the experiment from Day 7 to Day 84 inclusive.

Source	df	MS	F
Group (G)	3	4.71	5.37**
Subject (S)	26	.88	
Days (D)	11	.21	1.45
G x D	33	.28	1.96**
D x S	286	.14	

**p < .01.

TABLE 18

The analysis of variance of the comparison of the temperature of animals in all groups at 18 h (six hours after morphine administration) on Days 7, 14, and 21 and on Days 70, 77, and 84.

Source	df	MS	F
Group (G)	3	3.67	7.77**
Subject (S)	26	.47	
Block (B)	1	.75	4.62*
G x B	3	.97	5.97**
B x S	26	.16	
Days (D)	2	.12	.72
G x D	6	.07	.44
D x S	52	.16	
B x D	2	.01	.08
G x B x D	6	.22	1.35
B x D x S	52	.16	

*$p < .05$.

**$p < .01$.

TABLE 19

The analysis of variance of the temperature of animals in all groups at 11 h in the pre-injection environment for Days 6 to 9.

Source	df	MS	F
Group (G)	3	1.32	9.84**
Subject (S)	26	.13	
Days (D)	3	.78	11.46**
G x D	9	.27	4.03**
D x S	78	.07	

**$p < .01$.

TABLE 20

The analysis of variance of the temperature of animals in all groups at 11 h for every seventh day of the experiment from Day 7 to Day 84 inclusive.

Source	df	MS	F
Group (G)	3	16.66	56.57**
Subject (S)	26	.29	
Days (D)	11	1.55	15.35**
G x D	33	.25	2.46**
D x S	286	.10	

**$p < .01$.

TABLE 21

The analysis of variance of the comparison of the temperature of animals in all groups at 11 h on Days 7, 14, and 21 and Days 70, 77, and 84.

Source	df	MS	F
Group (G)	3	6.55	29.08**
Subject (S)	26	.23	
Block (B)	1	3.73	16.86**
G x B	3	1.40	6.35**
B x S	26	.22	
Days (D)	2	2.28	35.02**
G x D	6	.02	.32
D x S	52	.07	
B x D	2	.85	10.73**
G x B x D	6	.19	2.44*
B x D x S	52	.08	

*$p < .05$.
**$p < .01$.

TABLE 22

The analysis of variance of the comparison of the weights of all animals in all groups 21 hours and 45 hours after the last morphine administration for four pairs of days, Days 37,38; 43,44; 79,80; and 85,86.

Source	df	MS	F
Group (G)	3	199799.00	40.51**
Subject (S)	26	4931.56	
Time (T)	1	6303.75	45.30**
G x T	3	3105.22	22.31**
T x S	26	139.16	
Replication (R)	3	40040.40	330.84**
G x R	9	2201.51	18.19**
R x S	78	121.03	
T x R	3	79.31	.99
G x T x R	9	36.78	.46
T x R x S	78	79.72	

**$p < .01$.

TABLE 23

The analysis of variance of the temperature of animals in all groups 23 hours after the last morphine administration, at 11 h, in the pre-injection environment, for Days 37 and 79.

Source	df	MS	F
Group (G)	3	3.18	24.83**
Subject (S)	26	.13	
Replication (R)	1	2.02	26.51**
G x R	3	.23	2.96
R x S	26	.08	

**$p < .01$.

TABLE 24

The analysis of variance of the temperature of animals in all groups 47 hours after the last morphine administration, at 11 h, in the pre-injection environment for Days 38 and 80.

Source	df	MS	F
Group (G)	3	2.95	26.40**
Subject (S)	26	.11	
Replication (R)	1	.49	6.98*
G x R	3	.03	.44
R x S	26	.07	

*$p < .05$.

**$p < .01$.

TABLE 25

The analysis of variance of the temperature of animals in all groups 21 hours after the last morphine administration, at 9 h, in the home cage, for Days 37 and 79.

Source	df	MS	F
Group (G)	3	.05	.47
Subject (S)	26	.11	
Replication (R)	1	2.05	17.92**
G x R	3	.21	1.84
R x S	26	.11	

**$p < .01$.

TABLE 26

The analysis of variance of the temperature of animals in all groups 45 hours after the last morphine administration, at 9 h, in the home cage, for Days 38 and 80.

Source	df	MS	F
Group (G)	3	.88	4.26*
Subject (S)	26	.21	
Replication (R)	1	2.65	39.90**
G x R	3	.21	3.11*
R x S	26	.07	

*$p < .05$.
**$p < .01$.

TABLE 27

The analysis of variance of the comparison of the temperature of animals in all groups at 11 h on four days in the pre-injection environment, Days 37, 51, 65, and 74 and four days in the home cage, Days 43, 57, 71, and 85.

Source	df	MS	F
Groups (G)	3	7.10	23.92**
Subject (S)	26	.30	
Environment (E)	1	4.76	41.06**
G x E	3	.67	5.75**
E x S	26	.12	
Replication (R)	3	3.68	41.69**
G x R	9	.19	2.19*
R x S	78	.09	
E x R	3	.26	3.43*
G x E x R	9	.08	1.13
E x R x S	78	.07	

*$p < .05$.

**$p < .01$.

TABLE 28

The analysis of variance of the temperature of animals in all groups at 9 h on the first 5 days of abstinence, Days 85 to 89.

Source	df	MS	F
Group (G)	3	.58	1.45
Subject (S)	26	.40	
Days (D)	4	.34	3.17*
G x D	12	.12	1.14
D x S	104	.11	

*p < .05.

TABLE 29

The analysis of variance of the temperature of animals in all groups at 11 h on the first 5 days of abstinence, Days 85 to 89.

Source	df	MS	F
Group (G)	3	1.75	6,37**
Subject (S)	26	.27	
Days (D)	4	.38	3.49*
G x D	12	.17	1.58
D x S	104	.11	

*p < .05.

**p < .01.

TABLE 30

The analysis of variance of a comparison of the temperature of animals in all groups at 11 h on the first three days of abstinence, Days 85, 86, and 87 and the last three days of abstinence, Days 92, 93, and 94.

Source	df	MS	F
Group (G)	3	1.12	2.45
Subject (S)	26	.46	
Block (B)	1	.33	2.61
G x B	3	.63	5.03**
B x D	26	.13	
Days (D)	2	.24	1.94
G x D	6	.18	1.47
D x S	52	.13	
B x D	2	.41	3.10
G x B x D	6	.14	1.04
B x D x S	52	.13	

**$p < .01$.

TABLE 31

The analysis of variance of a comparison of the temperature of animals in all groups at 9 h on Days 94 and 120 and on Days 95 and 121.

Source	df	MS	F
Group (G)	3	.07	.13
Subject (S)	26	.53	
Environment (E)	1	4.93	36.43**
G x E	3	.11	.80
E x S	26	.14	
Replication (R)	1	10.03	44.78**
G x R	3	.12	.52
R x S	26	.22	
E x R	1	2.49	13.85**
G x E x R	3	.21	1.19
E x R x S	26	.18	

**$p < .01$.

TABLE 32

The analysis of variance of the comparison of the temperature of animals in all groups at 11 h on days in the home cage, Days 94 and 120 and days in the pre-injection environment, Days 95 and 121.

Source	df	MS	F
Group (G)	3	.04	.21
Subject (S)	26	.19	
Environment (E)	1	9.80	96.31**
G x E	3	.18	1.79
E x S	26	.10	
Replication (R)	1	2.16	19.76**
G x R	3	.20	1.81
R x S	26	.11	
E x R	1	2.00	15.73**
G x E x R	3	.11	.85
E x R x S	26	.13	

**$p < .01$.

TABLE 33

The analysis of variance of the temperature of animals in all groups at 11 h in the pre-injection environment on days 95 to 99:

Source	df	MS	F
Groups (G)	3	.21	1.18
Subject (S)	26	.18	
Days (D)	3	2.08	27.24**
G x D	9	.08	1.05
D x S	78	.08	

**p < .01.

TABLE 34

The analysis of variance of the comparison of the temperature of animals in all groups at 12:45 h on days in the home cage, Days 94 and 120 and days in the post-injection environment, Days 95 and 121.

Source	df	MS	F
Group (G)	3	.91	2.39
Subject (S)	26	.38	
Environment (E)	1	9.41	77.76**
G x E	3	.13	1.11
E x S	26	.12	
Replication (R)	1	1.78	16.71**
G x R	3	.05	.42
R x S	26	.11	
E x R	1	1.93	16.52**
G x E x R	3	.02	.20
E x R x S	26	.12	

**$p < .01$.

TABLE 35

The analysis of variance of the comparison of the temperature of animals in all groups at 14:15 h on days in the home cage, Days 94 and 120, and days in the post-injection environment, Days 95 and 121.

Source	df	MS	F
Group (G)	3	1.01	2.36
Subject (S)	26	.43	
Environment (E)	1	18.10	114.57**
G x E	3	.63	3.96*
E x S	26	.16	
Replication (R)	1	.24	2.15
G x R	3	.15	1.30
R x S	26	.11	
E x R	1	.10	.96
G x E x R	3	.12	1.21
E x R x S	26	.10	

*$p < .05$.

**$p < .01$.

TABLE 36

The analysis of variance of the comparison of the temperature of animals in all groups at 12:45 h on days in the post-injection environment, Days 37 and 79, and days in the home cage, Days 43 and 85.

Source	df	MS	F
Group (G)	3	.47	2.74
Subject (S)	26	.17	
Environment (E)	1	.05	.86
G x E	3	.38	6.81**
E x S	26	.06	
Replication (R)	1	4.56	126.09**
G x R	3	.03	.84
R x S	26	.04	
E x R	1	.10	2.22
G x E x R	3	.01	.31
E x R x S	26	.04	

**$p < .01$.

TABLE 37

The analysis of variance of the comparison of the temperature of animals in all groups at 14:15 h on days in the post-injection environment, Days 37 and 79, and days in the home cage, Days 43 and 85.

Source	df	MS	F
Group (G)	3	.48	3.29*
Subjects (S)	26	.15	
Environment (E)	1	.29	6.14*
G x E	3	.96	20.39**
E x S	26	.05	
Replication (R)	1	4.07	52.59**
G x R	3	.10	1.27
R x S	26	.08	
E x R	1	.33	2.90
G x E x R	3	.13	1.12
E x R x S	26	.11	

*$p < .05$.

**$p < .01$.

TABLE 38

The analysis of variance of the comparison of the temperature difference between 11 h and 12:45 h of animals in all groups in the conditioning environments during conditioning, Days 37 and 79, and after abstinence, Days 95 and 121.

Source	df	MS	F
Group (G)	3	2.20	29.61**
Subject (S)	26	.07	
Period (P)	1	.18	2.11
G x P	3	.03	.30
P x S	26	.09	
Replication (R)	1	.08	.98
G x R	3	.10	1.27
R x S	26	.08	
P x R	1	.03	.23
G x P x R	3	.08	.66
P x R x S	26	.12	

**$p < .01$.

TABLE 39

The analysis of variance of the comparison of the temperature difference between 11 h and 14:15 h of animals in all groups in the conditioning environments during conditioning, Days 37 and 79 and after abstinence, Days 95 and 121.

Source	df	MS	F
Group (G)	3	2.78	8.95**
Subject (S)	26	.31	
Period (P)	1	2.11	14.45**
G x P	3	.16	1.07
P x S	26	.15	
Replication (R)	1	.05	.49
G x R	3	.12	1.13
R x S	26	.11	
P x R	1	.13	1.15
G x P x R	3	.39	3.57*
P x R x S	26	.11	

*$p < .05$.

**$p < .01$.

TABLE 40

The analysis of variance of the comparison of the temperature of animals in all groups after abstinence, at 12:45 h, on a saline test day, Day 96 and a naloxone test day, Day 97.

Source	df	MS	F
Group (G)	3	1.02	6.06**
Subject (S)	26	.17	
Days (D)	1	7.49	72.98**
G x D	3	.03	.29
D x S	26	.10	

**$p < .01$.

TABLE 41

The analysis of variance of the comparison of the temperature of animals in all groups after abstinence, at 14:15 h, on a saline test day, Day 96, and on a naloxone test day, Day 97.

Source	df	MS	F
Group (G)	3	2.45	8.47**
Subject (S)	26	.29	
Days (D)	1	.40	3.29
G x D	3	.04	.34
D x S	26	.12	

**$p < .01$.

Appendix B

Means and standard deviations of body temperature (^{o}C) of animals in the four groups.

The following tables contain the group means and standard deviation for the five daily temperature readings. The first four tables are for the days in the home cage prior to the start of the experiment proper (Days i to iv). In the subsequent tables the daily temperature means and standard deviations for the days of the experiment proper are presented. The days refer to the day of the experiment and the number after the means refers to the Group size.

For the four days prior to the start of the experiment proper, the temperature readings were taken at 9 h, 12 h, 18 h, and at 21 h (Times 1 to 5 respectively).

	TIME	GROUP	1	2	3	4
Day i	1	MEAN	37.337 8	37.425 8	37.557 7	37.529 7
		S.D.	0.267	0.369	0.416	0.335
	2	MEAN	37.450 8	37.812 8	37.714 7	37.614 7
		S.D.	0.444	0.380	0.527	0.471
	3	MEAN	37.575 8	37.725 8	37.571 7	37.329 7
		S.D.	0.456	0.427	0.377	0.138
	4	MEAN	37.312 8	37.362 8	37.371 7	37.414 7
		S.D.	0.223	0.165	0.198	0.195
	5	MEAN	37.475 8	37.412 8	37.557 7	37.871 7
		S.D.	0.260	0.331	0.447	0.479
Day ii	1	MEAN	37.625 8	37.750 8	37.729 7	37.871 7
		S.D.	0.349	0.378	0.582	0.243
	2	MEAN	37.200 8	37.425 8	37.129 7	37.529 7
		S.D.	0.141	0.306	0.111	0.461
	3	MEAN	37.375 8	37.375 8	37.414 7	37.443 7
		S.D.	0.219	0.396	0.414	0.435
	4	MEAN	37.175 8	37.425 8	37.557 7	37.586 7
		S.D.	0.191	0.570	0.565	0.463
	5	MEAN	37.675 8	37.987 8	37.929 7	38.471 7
		S.D.	0.489	0.559	0.461	0.431
Day iii	1	MEAN	37.363 8	37.912 8	37.300 7	37.471 7
		S.D.	0.200	0.544	0.332	0.489
	2	MEAN	37.700 8	37.712 8	37.757 7	37.600 7
		S.D.	0.407	0.610	0.602	0.443
	3	MEAN	37.312 8	37.400 8	37.357 7	37.429 7
		S.D.	0.236	0.359	0.374	0.509
	4	MEAN	37.262 8	37.887 8	37.600 7	37.700 7
		S.D.	0.207	0.416	0.658	0.476
	5	MEAN	37.900 8	38.112 8	38.186 7	38.029 7
		S.D.	0.467	0.562	0.449	0.670

Day iv

TIME	GROUP	1	2	3	4
1	MEAN	37.062 8	37.350 8	37.314 7	37.300 7
	S.D.	0.119	0.330	0.567	0.436
2	MEAN	37.350 8	37.500 8	37.257 7	37.529 7
	S.D.	0.355	0.389	0.346	0.535
3	MEAN	37.150 8	37.275 8	37.286 7	37.243 7
	S.D.	0.131	0.328	0.254	0.172
4	MEAN	37.400 8	37.575 8	37.629 7	37.643 7
	S.D.	0.602	0.489	0.415	0.704
5	MEAN	38.150 8	38.513 8	38.371 7	38.500 7
	S.D.	0.605	0.360	0.461	0.432

During the experiment the temperature readings were taken at 9 h, 11 h, 12:45 h, 14:15 h, and 18 h (Times 1 to 5 respectively).

	TIME	GROUP	1	2	3	4
Day 1	1	MEAN	38.075	38.337	38.057	38.129
		S.D.	0.634	0.548	0.721	0.582
	2	MEAN	38.550	38.625	38.471	38.386
		S.D.	0.151	0.149	0.206	0.168
	3	MEAN	37.750	37.775	37.743	37.729
		S.D.	0.338	0.377	0.336	0.435
	4	MEAN	37.287	37.250	37.329	37.257
		S.D.	0.264	0.312	0.180	0.341
	5	MEAN	37.112	37.200	37.100	37.343
		S.D.	0.285	0.370	0.082	0.412
Day 2	1	MEAN	37.887	38.175	37.843	38.443
		S.D.	0.416	0.260	0.635	0.395
	2	MEAN	38.275	38.187	38.071	37.936
		S.D.	0.225	0.344	0.461	0.261
	3	MEAN	37.625	37.737	37.629	37.557
		S.D.	0.260	0.475	0.206	0.181
	4	MEAN	37.413	37.275	37.329	37.057
		S.D.	0.240	0.292	0.221	0.151
	5	MEAN	37.487	37.412	37.557	38.071
		S.D.	0.412	0.562	0.574	0.475
Day 3	1	MEAN	37.487	37.437	37.643	37.657
		S.D.	0.259	0.513	0.447	0.635
	2	MEAN	38.250	38.088	38.014	37.943
		S.D.	0.233	0.387	0.534	0.326
	3	MEAN	37.537	37.675	37.600	37.386
		S.D.	0.307	0.430	0.356	0.227
	4	MEAN	37.337	37.413	37.500	37.171
		S.D.	0.366	0.360	0.523	0.214
	5	MEAN	38.012	38.337	38.300	38.457
		S.D.	0.579	0.389	0.668	0.535

	TIME	GROUP	1	2	3	4
Day 4	1	MEAN	37.900	38.000	37.757	37.971
		S.D.	0.407	0.588	0.836	0.509
	2	MEAN	38.200	38.075	38.157	37.871
		S.D.	0.400	0.410	0.424	0.261
	3	MEAN	37.512	37.675	37.571	37.429
		S.D.	0.327	0.301	0.442	0.287
	4	MEAN	37.350	37.338	37.643	37.357
		S.D.	0.273	0.378	0.640	0.326
	5	MEAN	38.075	38.087	37.914	38.400
		S.D.	0.671	0.439	0.780	0.238
Day 5	1	MEAN	38.312 8	38.112 8	38.386 7	38.343 7
		S.D.	0.304	0.387	0.267	0.364
	2	MEAN	38.187 8	38.225 8	38.114 7	38.043 7
		S.D.	0.309	0.296	0.488	0.378
	3	MEAN	37.125 8	37.300 8	38.086 7	38.657 7
		S.D.	0.231	0.481	0.612	0.619
	4	MEAN	37.575 8	37.987 8	39.071 7	39.514 7
		S.D.	0.443	0.675	0.528	1.238
	5	MEAN	38.100 8	38.237 8	38.871 7	38.014 7
		S.D.	0.739	0.850	0.320	0.899
Day 6	1	MEAN	38.250 8	38.063 8	38.071 7	38.386 7
		S.D.	0.469	0.358	0.588	0.456
	2	MEAN	37.962 8	37.987 8	37.886 7	38.143 7
		S.D.	0.320	0.217	0.389	0.215
	3	MEAN	37.762 8	38.862 8	38.871 7	39.057 7
		S.D.	0.316	0.325	0.577	0.360
	4	MEAN	37.662 8	38.950 8	39.871 7	39.857 7
		S.D.	0.481	0.400	0.403	0.571
	5	MEAN	37.500 8	37.512 8	37.729 7	37.429 7
		S.D.	0.351	0.473	0.640	0.559

TIME	GROUP	1	2	3	4
Day 7					
1	MEAN	38.212 8	38.000 8	37.943 7	38.100 7
	S.D.	0.196	0.342	0.465	0.514
2	MEAN	38.325 8	37.900 8	37.943 7	37.857 7
	S.D.	0.238	0.302	0.360	0.159
3	MEAN	37.812 8	39.188 8	39.157 7	38.971 7
	S.D.	0.270	0.285	0.310	0.229
4	MEAN	37.512 8	39.000 8	39.986 7	39.929 7
	S.D.	0.485	0.239	0.406	0.275
5	MEAN	37.750 8	37.412 8	37.186 7	37.757 7
	S.D.	0.639	0.336	0.691	0.399
Day 8					
1	MEAN	38.087 8	37.950 8	38.143 7	37.929 7
	S.D.	0.405	0.370	0.387	0.355
2	MEAN	38.050 8	37.575 8	37.586 7	37.486 7
	S.D.	0.262	0.292	0.241	0.389
3	MEAN	37.537 8	39.237 8	38.914 7	38.414 7
	S.D.	0.421	0.329	0.273	0.438
4	MEAN	37.500 8	39.075 8	40.114 7	40.071 7
	S.D.	0.355	0.260	0.157	0.373
5	MEAN	38.012 8	37.312 8	37.457 7	37.671 7
	S.D.	0.488	0.432	0.616	0.461
Day 9					
1	MEAN	38.300 8	38.012 8	37.957 7	38.029 7
	S.D.	0.417	0.290	0.360	0.293
2	MEAN	38.312 8	37.637 8	37.371 7	37.757 7
	S.D.	0.223	0.277	0.180	0.431
3	MEAN	37.587 8	39.337 8	39.171 7	38.514 7
	S.D.	0.391	0.169	0.364	1.042
4	MEAN	37.587 8	39.087 8	39.871 7	39.886 7
	S.D.	0.318	0.189	0.482	0.825
5	MEAN	37.650 8	37.675 8	37.186 7	37.529 7
	S.D.	0.573	0.754	0.426	0.559

	TIME	GROUP	1	2	3	4
Day 10	1	MEAN	38.387 8	37.900 8	37.900 7	38.114 7
		S.D.	0.372	0.160	0.291	0.348
	2	MEAN	38.150 8	37.575 8	37.386 7	37.629 7
		S.D.	0.338	0.301	0.254	0.411
	3	MEAN	37.375 8	38.925 8	39.014 7	38.814 7
		S.D.	0.337	0.776	0.438	0.659
	4	MEAN	37.550 8	38.650 8	39.986 7	40.129 7
		S.D.	0.382	0.632	0.121	0.180
	5	MEAN	38.212 8	37.712 8	37.557 7	37.414 7
		S.D.	0.452	0.455	0.553	0.398
Day 11	1	MEAN	37.962 8	37.625 8	37.543 7	37.814 7
		S.D.	0.358	0.333	0.098	0.389
	2	MEAN	38.025 8	37.825 8	37.271 7	37.543 7
		S.D.	0.320	0.140	0.180	0.383
	3	MEAN	37.425 8	38.925 8	38.771 7	38.300 7
		S.D.	0.349	0.801	0.522	1.145
	4	MEAN	37.412 8	38.712 8	39.643 7	39.457 7
		S.D.	0.439	0.714	0.127	0.387
	5	MEAN	37.975 8	37.562 8	37.286 7	37.443 7
		S.D.	0.465	0.400	0.358	0.486
Day 12	1	MEAN	38.212 8	37.900 8	37.814 7	37.929 7
		S.D.	0.290	0.207	0.212	0.373
	2	MEAN	38.012 8	37.650 8	37.229 7	37.714 7
		S.D.	0.259	0.389	0.298	0.543
	3	MEAN	37.275 8	38.825 8	38.843 7	38.543 7
		S.D.	0.406	0.698	0.428	0.976
	4	MEAN	37.562 8	38.412 8	39.586 7	39.757 7
		S.D.	0.403	0.718	0.372	0.408
	5	MEAN	38.088 8	37.637 8	37.329 7	37.757 7
		S.D.	0.544	0.421	0.591	0.493

	TIME	GROUP	1	2	3	4
Day 13	1	MEAN	38.212 8	37.837 8	37.729 7	38.000 7
		S.D.	0.372	0.385	0.206	0.490
	2	MEAN	38.062 8	37.487 8	37.289 7	37.643 7
		S.D.	0.362	0.253	0.263	0.346
	3	MEAN	37.437 8	39.287 8	38.857 7	38.529 7
		S.D.	0.427	0.348	0.544	0.673
	4	MEAN	37.712 8	38.900 8	39.314 7	39.657 7
		S.D.	0.541	0.532	0.649	0.351
	5	MEAN	37.775 8	37.263 8	37.100 7	37.457 7
		S.D.	0.362	0.417	0.342	0.619
Day 14	1	MEAN	37.462 8	37.462 8	37.257 7	37.457 7
		S.D.	0.521	0.400	0.399	0.408
	2	MEAN	37.987 8	37.375 8	37.143 7	37.414 7
		S.D.	0.388	0.315	0.140	0.463
	3	MEAN	37.187 8	38.925 8	38.457 7	38.314 7
		S.D.	0.242	0.443	0.680	0.667
	4	MEAN	37.325 8	38.662 8	39.400 7	39.186 7
		S.D.	0.315	0.686	0.455	0.910
	5	MEAN	37.862 8	37.087 8	37.386 7	37.729 7
		S.D.	0.501	0.275	0.438	0.423
Day 15	1	MEAN	37.775 8	37.512 8	37.571 7	37.686 7
		S.D.	0.430	0.491	0.439	0.402
	2	MEAN	38.075 8	37.575 8	37.243 7	37.457 7
		S.D.	0.345	0.423	0.355	0.355
	3	MEAN	37.325 8	39.300 8	39.186 7	38.729 7
		S.D.	0.369	0.396	0.344	0.824
	4	MEAN	37.800 8	38.850 8	39.571 7	39.743 7
		S.D.	0.481	0.561	0.368	0.378
	5	MEAN	37.550 8	37.137 8	37.200 7	37.443 7
		S.D.	0.616	0.245	0.480	0.538

	TIME	GROUP	1	2	3	4
Day 16	1	MEAN	38.325 8	37.712 8	37.729 7	37.729 7
		S.D.	0.396	0.264	0.275	0.427
	2	MEAN	38.125 8	37.150 8	37.014 7	37.186 7
		S.D.	0.337	0.093	0.157	0.273
	3	MEAN	37.212 8	39.125 8	38.800 7	38.900 7
		S.D.	0.259	0.282	0.952	0.503
	4	MEAN	37.412 8	38.862 8	39.557 7	39.786 7
		S.D.	0.439	0.748	0.244	0.414
	5	MEAN	37.712 8	37.212 8	37.314 7	37.900 7
		S.D.	0.422	0.591	0.612	0.572
Day 17	1	MEAN	37.800 8	37.650 8	37.300 7	37.543 7
		S.D.	0.605	0.283	0.392	0.351
	2	MEAN	37.800 8	37.300 8	37.143 7	37.100 7
		S.D.	0.298	0.342	0.355	0.294
	3	MEAN	36.900 8	39.225 8	39.100 7	38.286 7
		S.D.	0.251	0.301	0.503	0.587
	4	MEAN	37.462 8	38.963 8	39.471 7	39.629 7
		S.D.	0.403	0.316	0.355	0.442
	5	MEAN	37.437 8	36.875 8	37.186 7	37.243 7
		S.D.	0.650	0.225	0.641	0.299
Day 18	1	MEAN	37.837 8	37.750 8	37.414 7	37.429 7
		S.D.	0.573	0.330	0.376	0.457
	2	MEAN	37.987 8	37.162 8	37.086 7	37.157 7
		S.D.	0.372	0.329	0.329	0.172
	3	MEAN	37.400 8	39.137 8	38.657 7	39.200 7
		S.D.	0.239	0.338	0.591	0.443
	4	MEAN	37.525 8	39.125 8	39.743 7	39.829 7
		S.D.	0.198	0.287	0.454	0.335
	5	MEAN	37.887 8	36.975 8	37.214 7	37.929 7
		S.D.	0.603	0.396	0.291	0.626

	TIME	GROUP	1	2	3	4
Day 19	1	MEAN	38.075 8	37.687 8	37.529 7	37.657 7
		S.D.	0.486	0.210	0.293	0.486
	2	MEAN	37.987 8	37.338 8	36.943 7	37.057 7
		S.D.	0.506	0.407	0.140	0.181
	3	MEAN	37.250 8	39.100 8	38.943 7	38.386 7
		S.D.	0.346	0.346	0.408	0.576
	4	MEAN	37.025 8	38.650 8	39.471 7	39.257 7
		S.D.	0.385	0.605	0.150	0.465
	5	MEAN	37.350 8	36.888 8	37.171 7	37.400 7
		S.D.	0.682	0.442	0.439	0.792
Day 20	1	MEAN	37.687 8	37.475 8	37.329 7	37.200 7
		S.D.	0.419	0.212	0.395	0.342
	2	MEAN	37.875 8	37.287 8	36.971 7	37.214 7
		S.D.	0.547	0.331	0.205	0.353
	3	MEAN	37.150 8	39.162 8	39.200 7	38.186 7
		S.D.	0.338	0.141	0.523	0.788
	4	MEAN	37.412 8	38.925 8	39.700 7	39.714 7
		S.D.	0.429	0.128	0.440	0.273
	5	MEAN	1.000 2	2.000 2	3.000 2	4.000 2
		S.D.	0	0	0	0
Day 21	1	MEAN	37.662 8	37.212 8	37.271 7	37.529 7
		S.D.	0.493	0.280	0.206	0.214
	2	MEAN	38.100 8	37.475 8	37.071 7	37.457 7
		S.D.	0.393	0.403	0.095	0.336
	3	MEAN	37.162 8	39.113 8	38.886 7	38.586 7
		S.D.	0.256	0.136	0.659	0.438
	4	MEAN	37.575 8	38.837 8	39.557 7	39.871 7
		S.D.	0.560	0.431	0.461	0.395
	5	MEAN	37.862 8	37.150 8	37.229 7	37.414 7
		S.D.	0.657	0.351	0.243	0.501

	TIME	GROUP	1	2	3	4
Day 22	1	MEAN	37.987 8	37.462 8	37.500 7	37.871 7
		S.D.	0.439	0.475	0.428	0.403
	2	MEAN	37.975 8	37.200 8	37.029 7	37.200 7
		S.D.	0.403	0.177	0.180	0.300
	3	MEAN	37.262 8	39.037 8	39.243 7	38.486 7
		S.D.	0.320	0.637	0.399	0.639
	4	MEAN	37.587 8	38.950 8	39.800 7	39.857 7
		S.D.	0.323	0.396	0.289	0.486
	5	MEAN	37.850 8	37.600 8	37.771 7	37.614 7
		S.D.	0.571	0.532	0.450	0.422
Day 23	1	MEAN	37.637 8	37.112 8	37.429 7	37.357 7
		S.D.	0.644	0.210	0.368	0.199
	2	MEAN	38.150 8	37.537 8	37.671 7	37.700 7
		S.D.	0.293	0.141	0.236	0.183
	3	MEAN	37.362 8	39.175 8	39.043 7	38.886 7
		S.D.	0.329	0.406	0.472	0.564
	4	MEAN	37.500 8	38.975 8	39.729 7	40.071 7
		S.D.	0.267	0.333	0.515	0.403
	5	MEAN	37.875 8	37.350 8	37.700 7	37.514 7
		S.D.	0.495	0.378	0.374	0.227
Day 24	1	MEAN	37.937 8	37.350 8	37.500 7	37.629 7
		S.D.	0.484	0.245	0.332	0.189
	2	MEAN	38.187 8	37.500 8	37.171 7	37.229 7
		S.D.	0.562	0.278	0.256	0.320
	3	MEAN	37.425 8	39.325 8	39.214 7	38.757 7
		S.D.	0.437	0.392	0.720	0.435
	4	MEAN	37.587 8	39.075 8	39.457 7	39.814 7
		S.D.	0.383	0.231	0.435	0.367
	5	MEAN	37.937 8	37.562 8	37.829 7	37.529 7
		S.D.	0.475	0.414	0.624	0.577

	TIME	GROUP	1	2	3	4
Day 25	1	MEAN	38.050 8	37.750 8	37.386 7	37.414 7
		S.D.	0.382	0.346	0.380	0.302
	2	MEAN	38.100 8	37.350 8	37.086 7	37.414 7
		S.D.	0.283	0.233	0.334	0.521
	3	MEAN	37.237 8	39.162 8	39.186 7	38.757 7
		S.D.	0.320	0.346	0.491	0.574
	4	MEAN	37.537 8	39.125 8	39.786 7	39.871 7
		S.D.	0.447	0.282	0.157	0.281
	5	MEAN	37.787 8	37.325 8	37.643 7	37.414 7
		S.D.	0.544	0.255	0.655	0.219
Day 26	1	MEAN	37.862 8	37.562 8	37.629 7	37.786 7
		S.D.	0.568	0.325	0.325	0.267
	2	MEAN	38.262 8	37.500 8	37.329 7	37.429 7
		S.D.	0.213	0.256	0.170	0.568
	3	MEAN	37.187 8	39.312 8	39.229 7	38.771 7
		S.D.	0.275	0.181	0.350	0.395
	4	MEAN	37.500 8	39.025 8	39.714 7	40.014 7
		S.D.	0.273	0.205	0.186	0.313
	5	MEAN	37.812 8	37.338 8	37.600 7	37.700 7
		S.D.	0.500	0.262	0.400	0.705
Day 27	1	MEAN	37.800 8	37.737 8	37.514 7	37.457 7
		S.D.	0.507	0.220	0.376	0.276
	2	MEAN	38.312 8	37.600 8	37.171 7	37.100 7
		S.D.	0.223	0.404	0.221	0.129
	3	MEAN	37.662 8	39.450 8	39.271 7	39.229 7
		S.D.	0.424	0.131	0.411	0.359
	4	MEAN	37.725 8	39.125 8	39.986 7	39.771 7
		S.D.	0.518	0.271	0.463	0.298
	5	MEAN	37.375 8	37.487 8	37.986 7	38.057 7
		S.D.	0.276	0.442	0.715	0.695

	TIME	GROUP	1	2	3	4
Day 28	1	MEAN	37.462 8	37.162 8	37.129 7	37.257 7
		S.D.	0.396	0.267	0.320	0.305
	2	MEAN	37.850 8	37.612 8	37.029 7	37.114 7
		S.D.	0.466	0.275	0.125	0.121
	3	MEAN	37.263 8	39.175 8	38.986 7	38.414 7
		S.D.	0.407	0.301	0.372	0.313
	4	MEAN	37.475 8	39.012 8	40.029 7	40.100 7
		S.D.	0.430	0.223	0.111	0.392
	5	MEAN	37.512 8	37.462 8	37.629 7	37.500 7
		S.D.	0.479	0.245	0.382	0.548
Day 29	1	MEAN	37.737 8	37.412 8	37.214 7	37.357 7
		S.D.	0.350	0.300	0.273	0.237
	2	MEAN	38.000 8	37.475 8	37.157 7	36.986 7
		S.D.	0.431	0.377	0.326	0.195
	3	MEAN	37.450 8	39.175 8	39.086 7	38.629 7
		S.D.	0.447	0.358	0.644	0.439
	4	MEAN	37.737 8	39.012 8	39.971 7	39.900 7
		S.D.	0.553	0.295	0.263	0.265
	5	MEAN	37.662 8	37.625 8	37.729 7	38.186 7
		S.D.	0.605	0.453	0.634	0.669
Day 30	1	MEAN	37.550 8	37.263 8	37.229 7	37.600 7
		S.D.	0.507	0.262	0.256	0.342
	2	MEAN	38.337 8	38.025 8	37.386 7	37.300 7
		S.D.	0.213	0.219	0.297	0.412
	3	MEAN	37.500 8	39.162 8	39.329 7	39.029 7
		S.D.	0.302	0.623	0.399	0.442
	4	MEAN	37.525 8	39.075 8	40.143 7	39.971 7
		S.D.	0.489	0.354	0.140	0.411
	5	MEAN	38.087 8	37.550 8	37.443 7	37.671 7
		S.D.	0.662	0.411	0.276	0.675

	TIME	GROUP	1	2	3	4
Day 31	1	MEAN	37.662 8	37.562 8	37.429 7	37.286 7
		S.D.	0.393	0.444	0.350	0.297
	2	MEAN	38.212 8	37.625 8	37.100 7	37.071 7
		S.D.	0.230	0.462	0.200	0.180
	3	MEAN	37.312 8	39.237 8	39.243 7	38.929 7
		S.D.	0.376	0.350	0.288	0.320
	4	MEAN	37.762 8	39.050 8	40.143 7	39.871 7
		S.D.	0.424	0.382	0.151	0.492
	5	MEAN	37.862 8	37.587 8	37.629 7	37.986 7
		S.D.	0.607	0.482	0.359	0.662
Day 32	1	MEAN	37.600	37.375	37.357	37.486
		S.D.	0.457	0.420	0.346	0.302
	2	MEAN	38.212	37.712	37.414	37.086
		S.D.	0.210	0.300	0.241	0.146
	3	MEAN	37.550	38.912	39.614	38.043
		S.D.	0.563	0.810	0.367	0.428
	4	MEAN	37.525	38.825	39.786	40.157
		S.D.	0.362	0.480	0.363	0.207
	5	MEAN	37.850	37.512	37.529	37.586
		S.D.	0.721	0.368	0.214	0.715
Day 33	1	MEAN	38.025	37.787	37.400	37.557
		S.D.	0.498	0.452	0.265	0.341
	2	MEAN	38.250	37.762	37.014	37.043
		S.D.	0.251	0.262	0.146	0.190
	3	MEAN	37.225	39.400	39.129	38.929
		S.D.	0.249	0.227	0.335	0.206
	4	MEAN	37.550	39.137	39.814	39.800
		S.D.	0.351	0.226	0.445	0.321
	5	MEAN	37.675	37.150	37.671	37.871
		S.D.	0.711	0.441	0.475	0.685

	TIME	GROUP	1	2	3	4
Day 34	1	MEAN	37.525 8	37.300 8	37.343 7	37.400 7
		S.D.	0.512	0.400	0.294	0.238
	2	MEAN	38.275 8	37.700 8	37.486 7	37.486 7
		S.D.	0.175	0.404	0.227	0.518
	3	MEAN	37.587 8	39.100 8	39.657 7	39.314 7
		S.D.	0.511	0.438	0.355	0.406
	4	MEAN	37.775 8	38.750 8	39.586 7	39.557 7
		S.D.	0.534	0.466	0.380	0.369
	5	MEAN	37.400 8	37.300 8	37.486 7	37.829 7
		S.D.	0.441	0.407	0.248	0.767
Day 35	1	MEAN	37.450 8	37.300 8	37.143 7	37.343 7
		S.D.	0.273	0.346	0.355	0.181
	2	MEAN	38.087 8	37.575 8	37.200 7	37.157 7
		S.D.	0.348	0.430	0.294	0.151
	3	MEAN	37.150 8	39.300 8	39.486 7	39.186 7
		S.D.	0.262	0.298	0.393	0.339
	4	MEAN	37.537 8	39.050 8	39.857 7	39.786 7
		S.D.	0.447	0.141	0.172	0.368
	5	MEAN	37.450 8	37.175 8	37.357 7	37.543 7
		S.D.	0.605	0.149	0.412	0.331
Day 36	1	MEAN	37.650 8	37.413 8	37.500 7	37.529 7
		S.D.	0.529	0.270	0.224	0.390
	2	MEAN	38.187 8	37.462 8	37.186 7	37.243 7
		S.D.	0.295	0.329	0.234	0.351
	3	MEAN	37.712 8	38.812 8	39.600 7	39.414 7
		S.D.	0.336	0.762	0.560	0.478
	4	MEAN	37.650 8	38.762 8	39.914 7	39.914 7
		S.D.	0.487	0.625	0.297	0.204
	5	MEAN	37.325 8	37.225 8	37.471 7	38.343 7
		S.D.	0.450	0.183	0.382	0.621

	TIME	GROUP	1	2	3	4
Day 37	1	MEAN	37.637 8	37.475 8	37.386 7	37.486 7
		S.D.	0.540	0.271	0.279	0.267
	2	MEAN	38.350 8	37.750 8	37.443 7	37.257 7
		S.D.	0.169	0.293	0.207	0.611
	3	MEAN	37.612 8	37.700 8	37.243 7	37.257 7
		S.D.	0.340	0.307	0.190	0.223
	4	MEAN	37.737 8	37.725 8	37.243 7	37.057 7
		S.D.	0.507	0.406	0.360	0.181
	5	MEAN	37.363 8	37.662 8	38.114 7	38.271 7
		S.D.	0.532	0.427	0.422	0.263
Day 38	1	MEAN	37.687 8	37.225 8	37.457 7	38.014 7
		S.D.	0.491	0.260	0.288	0.334
	2	MEAN	38.050 8	38.088 8	37.643 7	37.200 7
		S.D.	0.298	0.314	0.244	0.271
	3	MEAN	37.562 8	39.400 8	39.871 7	38.571 7
		S.D.	0.288	0.472	0.559	0.670
	4	MEAN	37.200 8	39.212 8	40.114 7	39.771 7
		S.D.	0.200	0.309	0.297	0.355
	5	MEAN	37.687 8	37.250 8	37.857 7	38.857 7
		S.D.	0.596	0.424	0.735	0.299
Day 39	1	MEAN	37.537 8	37.600 8	37.514 7	37.871 7
		S.D.	0.545	0.239	0.453	0.340
	2	MEAN	38.125 8	37.837 8	37.586 7	38.086 7
		S.D.	0.311	0.288	0.285	0.380
	3	MEAN	37.537 8	39.312 8	39.386 7	39.386 7
		S.D.	0.288	0.247	0.456	0.527
	4	MEAN	37.575 8	39.125 8	39.829 7	39.786 7
		S.D.	0.362	0.292	0.236	0.334
	5	MEAN	37.587 8	37.312 8	37.314 7	37.797 7
		S.D.	0.669	0.429	0.385	0.315

	TIME	GROUP	1	2	3	4
Day 40	1	MEAN	37.900 8	37.450 8	37.671 7	37.700 7
		S.D.	0.421	0.207	0.364	0.283
	2	MEAN	38.075 8	37.725 8	37.314 7	37.314 7
		S.D.	0.597	0.282	0.195	0.248
	3	MEAN	37.600 8	39.225 8	38.986 7	39.429 7
		S.D.	0.466	0.515	0.684	0.492
	4	MEAN	37.675 8	38.962 8	39.386 7	39.757 7
		S.D.	0.354	0.311	0.596	0.382
	5	MEAN	37.475 8	37.162 8	37.757 7	38.271 7
		S.D.	0.523	0.262	0.688	0.390
Day 41	1	MEAN	37.937 8	37.550 8	37.571 7	37.543 7
		S.D.	0.450	0.396	0.411	0.190
	2	MEAN	38.337 8	37.662 8	37.500 7	37.343 7
		S.D.	0.239	0.226	0.379	0.184
	3	MEAN	37.637 8	39.350 8	39.700 7	39.014 7
		S.D.	0.106	0.160	0.321	0.534
	4	MEAN	37.575 8	39.050 8	39.971 7	39.871 7
		S.D.	0.565	0.239	0.345	0.439
	5	MEAN	37.787 8	37.137 8	37.329 7	37.229 7
		S.D.	0.694	0.288	0.486	0.359
Day 42	1	MEAN	37.800 8	37.587 8	37.443 7	37.257 7
		S.D.	0.563	0.442	0.420	0.321
	2	MEAN	38.262 8	37.788 8	37.171 7	37.000 7
		S.D.	0.267	0.236	0.355	0.191
	3	MEAN	37.487 8	39.188 8	39.143 7	39.357 7
		S.D.	0.591	0.376	0.562	0.597
	4	MEAN	37.400 8	38.987 8	39.800 7	39.614 7
		S.D.	0.312	0.259	0.316	0.344
	5	MEAN	37.225 8	37.187 8	37.243 7	37.786 7
		S.D.	0.565	0.494	0.315	0.631

	TIME	GROUP	1	2	3	4
Day 43	1	MEAN	38.000 8	37.837 8	37.486 7	37.514 7
		S.D.	0.441	0.320	0.212	0.334
	2	MEAN	38.450 8	38.262 8	38.014 7	37.529 7
		S.D.	0.227	0.297	0.227	0.439
	3	MEAN	37.537 8	37.412 8	37.386 7	37.457 7
		S.D.	0.396	0.223	0.177	0.230
	4	MEAN	37.387 8	37.450 8	37.314 7	37.671 7
		S.D.	0.352	0.293	0.221	0.320
	5	MEAN	37.675 8	37.687 8	37.800 7	38.171 7
		S.D.	0.658	0.318	0.224	0.315
Day 44	1	MEAN	38.112 8	37.800 8	37.671 7	37.871 7
		S.D.	0.412	0.346	0.198	0.364
	2	MEAN	38.662 8	38.362 8	37.700 7	38.957 7
		S.D.	0.213	0.213	0.346	0.287
	3	MEAN	38.275 8	39.637 8	39.571 7	38.571 7
		S.D.	0.333	0.226	0.881	0.359
	4	MEAN	38.050 8	39.325 8	39.886 7	39.757 7
		S.D.	0.637	0.183	0.445	0.326
	5	MEAN	37.237 8	37.062 8	38.043 7	38.786 7
		S.D.	0.498	0.250	0.761	0.254
Day 45	1	MEAN	37.962 8	37.537 8	37.386 7	37.843 7
		S.D.	0.553	0.329	0.441	0.591
	2	MEAN	38.487 8	38.000 8	37.729 7	37.971 7
		S.D.	0.304	0.283	0.320	0.640
	3	MEAN	37.725 8	39.350 8	39.671 7	39.286 7
		S.D.	0.287	0.293	0.468	0.376
	4	MEAN	37.837 8	39.037 8	39.914 7	39.714 7
		S.D.	0.481	0.169	0.212	0.445
	5	MEAN	37.263 8	37.125 8	37.086 7	37.300 7
		S.D.	0.233	0.266	0.204	0.356

	TIME	GROUP	1	2	3	4
Day 46	1	MEAN	37.550 8	37.312 8	37.443 7	37.543 7
		S.D.	0.389	0.402	0.113	0.181
	2	MEAN	38.225 8	37.675 8	37.243 7	37.157 7
		S.D.	0.306	0.377	0.[illegible]0	0.150
	3	MEAN	37.562 8	39.275 8	39.171 7	39.286 7
		S.D.	0.245	0.337	0.709	0.618
	4	MEAN	37.712 8	39.062 8	39.743 7	39.886 7
		S.D.	0.402	0.297	0.529	0.398
	5	MEAN	37.662 8	37.200 8	37.700 7	37.714 7
		S.D.	0.524	0.463	0.762	0.308
Day 47	1	MEAN	37.775 8	37.537 8	37.357 7	37.657 7
		S.D.	0.609	0.524	0.399	0.408
	2	MEAN	38.112 8	37.537 8	37.284 7	37.200 7
		S.D.	0.173	0.385	0.204	0.300
	3	MEAN	37.263 8	39.125 8	38.971 7	38.557 7
		S.D.	0.262	0.175	0.632	0.387
	4	MEAN	37.525 8	38.962 8	39.757 7	39.529 7
		S.D.	0.492	0.262	0.151	0.281
	5	MEAN	38.075 8	37.187 8	37.429 7	37.514 7
		S.D.	0.616	0.360	0.340	0.348
Day 48	1	MEAN	37.787 8	37.612 8	37.486 7	37.471 7
		S.D.	0.497	0.360	0.146	0.365
	2	MEAN	38.000 8	37.787 8	37.071 7	36.957 7
		S.D.	0.293	0.242	0.298	0.190
	3	MEAN	37.125 8	38.962 8	38.957 7	38.714 7
		S.D.	0.231	0.469	0.461	0.771
	4	MEAN	37.412 8	38.875 8	39.471 7	39.600 7
		S.D.	0.636	0.373	0.243	0.374
	5	MEAN	37.775 8	37.362 8	37.829 7	37.571 7
		S.D.	0.570	0.431	0.565	0.281

	TIME	GROUP	1	2	3	4
Day 49	1	MEAN	37.637 8	37.487 8	37.286 7	37.529 7
		S.D.	0.595	0.511	0.302	0.442
	2	MEAN	38.200 8	37.687 8	37.071 7	36.814 7
		S.D.	0.316	0.452	0.250	0.273
	3	MEAN	37.362 8	39.162 8	38.943 7	38.986 7
		S.D.	0.466	0.460	0.237	0.334
	4	MEAN	37.412 8	39.162 8	39.671 7	39.457 7
		S.D.	0.470	0.177	0.189	0.399
	5	MEAN	37.700 8	37.262 8	37.586 7	37.843 7
		S.D.	0.670	0.292	0.453	0.472
Day 50	1	MEAN	37.125 8	37.212 8	37.414 7	37.186 7
		S.D.	0.385	0.331	0.297	0.227
	2	MEAN	37.437 8	37.375 8	37.257 7	36.814 7
		S.D.	0.466	0.496	0.101	0.212
	3	MEAN	37.050 8	39.062 8	39.000 7	38.900 7
		S.D.	0.256	0.487	0.583	0.726
	4	MEAN	37.400 8	39.050 8	39.757 7	39.514 7
		S.D.	0.501	0.245	0.310	0.558
	5	MEAN	37.812 8	37.050 8	37.357 7	37.657 7
		S.D.	0.608	0.131	0.315	0.321
Day 51	1	MEAN	37.700 8	37.425 8	37.443 7	37.329 7
		S.D.	0.515	0.489	0.360	0.198
	2	MEAN	37.800 8	37.500 8	37.029 7	36.729 7
		S.D.	0.518	0.466	0.236	0.138
	3	MEAN	36.950 8	36.875 8	36.814 7	36.300 7
		S.D.	0.417	0.377	0.168	0.821
	4	MEAN	37.200 8	37.012 8	37.071 7	36.414 7
		S.D.	0.293	0.318	0.198	0.438
	5	MEAN	38.025 8	38.037 8	37.671 7	37.886 7
		S.D.	0.673	0.463	0.095	0.234

	TIME	GROUP	1	2	3	4
Day 52	1	MEAN	36.737 8	36.812 8	37.257 7	37.357 7
		S.D.	0.403	0.196	0.244	0.399
	2	MEAN	37.562 8	37.425 8	37.343 7	37.029 7
		S.D.	0.490	0.520	0.270	0.340
	3	MEAN	37.187 8	38.975 8	39.071 7	38.657 7
		S.D.	0.327	0.462	0.824	0.424
	4	MEAN	37.450 8	38.787 8	40.057 7	39.614 7
		S.D.	0.454	0.799	0.230	0.463
	5	MEAN	37.713 8	36.950 8	37.743 7	38.243 7
		S.D.	0.514	0.245	0.416	0.574
Day 53	1	MEAN	37.475 8	37.350 8	37.371 7	37.314 7
		S.D.	0.632	0.374	0.457	0.329
	2	MEAN	37.725 8	37.537 8	37.571 7	37.443 7
		S.D.	0.599	0.659	0.390	0.321
	3	MEAN	37.062 8	38.537 8	39.200 7	39.114 7
		S.D.	0.316	0.986	0.906	0.438
	4	MEAN	37.187 8	38.500 8	39.243 7	39.071 7
		S.D.	0.348	0.351	0.447	0.475
	5	MEAN	37.862 8	36.975 8	37.143 7	37.800 7
		S.D.	0.697	0.276	0.331	0.283
Day 54	1	MEAN	37.387 8	37.350 8	37.386 7	37.171 7
		S.D.	0.755	0.421	0.204	0.320
	2	MEAN	37.787 8	37.412 8	37.157 7	37.314 7
		S.D.	0.488	0.314	0.310	0.540
	3	MEAN	37.038 8	39.050 8	39.071 7	39.043 7
		S.D.	0.389	0.363	0.315	0.435
	4	MEAN	37.137 8	38.925 8	39.286 7	39.386 7
		S.D.	0.250	0.205	0.219	0.422
	5	MEAN	37.725 8	36.912 8	37.057 7	37.786 7
		S.D.	0.592	0.500	0.374	0.555

	TIME	GROUP	1	2	3	4
Day 55	1	MEAN	37.750 8	37.263 8	37.129 7	37.457 7
		S.D.	0.630	0.329	0.243	0.351
	2	MEAN	37.750 8	37.175 8	36.971 7	37.014 7
		S.D.	0.393	0.399	0.198	0.241
	3	MEAN	37.000 8	39.150 8	38.929 7	39.086 7
		S.D.	0.302	0.267	0.640	0.543
	4	MEAN	37.275 8	38.850 8	39.386 7	39.529 7
		S.D.	0.453	0.233	0.460	0.399
	5	MEAN	37.412 8	36.950 8	37.386 7	37.443 7
		S.D.	0.567	0.214	0.501	0.230
Day 56	1	MEAN	37.100 8	36.888 8	36.943 7	37.043 7
		S.D.	0.450	0.295	0.215	0.199
	2	MEAN	37.550 8	37.137 8	36.814 7	36.743 7
		S.D.	0.563	0.510	0.241	0.162
	3	MEAN	36.888 8	38.925 8	38.986 7	38.414 7
		S.D.	0.253	0.266	0.581	0.628
	4	MEAN	37.150 8	38.912 8	39.414 7	39.429 7
		S.D.	0.370	0.247	0.212	0.198
	5	MEAN	37.788 8	37.162 8	37.314 7	37.486 7
		S.D.	0.394	0.378	0.380	0.267
Day 57	1	MEAN	37.225 8	36.950 8	37.000 7	37.014 7
		S.D.	0.337	0.389	0.294	0.254
	2	MEAN	37.775 8	37.537 8	37.286 7	37.114 7
		S.D.	0.570	0.329	0.279	0.212
	3	MEAN	36.875 8	37.025 8	36.886 7	36.371 7
		S.D.	0.183	0.292	0.135	0.763
	4	MEAN	36.962 8	37.212 8	37.143 7	36.871 7
		S.D.	0.185	0.146	0.162	0.650
	5	MEAN	37.450 8	37.775 8	37.757 7	37.714 7
		S.D.	0.581	0.531	0.264	0.353

	TIME	GROUP	1	2	3	4
Day 58	1	MEAN	36.900 8	37.012 8	37.171 7	37.571 7
		S.D.	0.370	0.223	0.377	0.281
	2	MEAN	38.225 8	37.762 8	37.486 7	36.957 7
		S.D.	0.249	0.481	0.376	0.428
	3	MEAN	37.600 8	39.188 8	39.400 7	38.686 7
		S.D.	0.307	0.467	0.980	0.567
	4	MEAN	37.512 8	39.037 8	39.643 7	39.571 7
		S.D.	0.528	0.417	0.387	0.287
	5	MEAN	36.862 8	36.825 8	37.957 7	38.414 7
		S.D.	0.185	0.149	0.675	0.515
Day 59	1	MEAN	37.375 8	37.225 8	37.086 7	37.186 7
		S.D.	0.595	0.373	0.418	0.157
	2	MEAN	37.600 8	37.575 8	37.286 7	37.586 7
		S.D.	0.363	0.396	0.453	0.456
	3	MEAN	36.912 8	38.687 8	38.929 7	39.071 7
		S.D.	0.203	0.766	0.519	0.330
	4	MEAN	37.700 8	38.737 8	39.357 7	39.286 7
		S.D.	0.338	0.644	0.172	0.456
	5	MEAN	37.675 8	37.387 8	37.229 7	37.986 7
		S.D.	0.713	0.467	0.298	0.426
Day 60	1	MEAN	37.475 8	37.000 8	37.071 7	37.129 7
		S.D.	0.565	0.293	0.345	0.229
	2	MEAN	37.650 8	37.375 8	37.086 7	37.043 7
		S.D.	0.535	0.423	0.318	0.215
	3	MEAN	36.900 8	38.762 8	38.686 7	38.557 7
		S.D.	0.330	0.628	0.558	0.443
	4	MEAN	37.100 8	38.800 8	39.306 7	39.500 7
		S.D.	0.245	0.256	0.372	0.366
	5	MEAN	37.825 8	37.175 8	37.529 7	37.800 7
		S.D.	0.547	0.231	0.306	0.523

	TIME	GROUP	1	2	3	4
Day 61	1	MEAN	37.700 8	37.500 8	37.400 7	37.443 7
		S.D.	0.586	0.605	0.306	0.341
	2	MEAN	37.637 8	37.400 8	37.157 7	36.857 7
		S.D.	0.524	0.312	0.276	0.321
	3	MEAN	36.800 8	38.737 8	38.729 7	38.643 7
		S.D.	0.256	0.393	0.320	0.613
	4	MEAN	37.137 8	38.537 8	39.271 7	39.529 7
		S.D.	0.417	0.555	0.150	0.479
	5	MEAN	37.662 8	37.200 8	37.343 7	37.529 7
		S.D.	0.447	0.256	0.412	0.325
Day 62	1	MEAN	37.562 8	37.475 8	37.286 7	37.414 7
		S.D.	0.550	0.498	0.463	0.508
	2	MEAN	38.087 8	37.525 8	37.029 7	36.729 7
		S.D.	0.247	0.276	0.214	0.269
	3	MEAN	37.025 8	39.225 8	39.100 7	39.100 7
		S.D.	0.296	0.243	0.557	0.705
	4	MEAN	37.175 8	38.925 8	39.057 7	39.600 7
		S.D.	0.437	0.116	0.562	0.473
	5	MEAN	37.425 8	37.037 8	37.743 7	37.657 7
		S.D.	0.456	0.325	0.522	0.544
Day 63	1	MEAN	37.300 8	37.350 8	37.014 7	37.186 7
		S.D.	0.424	0.414	0.449	0.426
	2	MEAN	37.750 8	37.625 8	36.943 7	36.771 7
		S.D.	0.490	0.260	0.326	0.236
	3	MEAN	37.075 8	39.100 8	38.514 7	38.586 7
		S.D.	0.477	0.251	0.749	0.636
	4	MEAN	36.925 8	38.750 8	38.929 7	39.571 7
		S.D.	0.385	0.302	0.419	0.468
	5	MEAN	37.550 8	36.987 8	37.657 7	37.600 7
		S.D.	0.540	0.242	0.479	0.440

	TIME	GROUP	1	2	3	4
Day 64	1	MEAN	37.437 8	37.600 8	37.329 7	37.400 7
		S.D.	0.616	0.444	0.287	0.231
	2	MEAN	37.962 8	37.375 8	37.071 7	36.700 7
		S.D.	0.277	0.243	0.304	0.141
	3	MEAN	37.112 8	39.212 8	39.114 7	38.800 7
		S.D.	0.295	0.173	0.488	0.231
	4	MEAN	37.275 8	38.950 8	39.657 7	39.871 7
		S.D.	0.486	0.160	0.282	0.298
	5	MEAN	37.500 8	37.112 8	37.429 7	37.357 7
		S.D.	0.590	0.196	0.640	0.479
Day 65	1	MEAN	37.475 8	37.350 8	37.229 7	37.271 7
		S.D.	0.757	0.288	0.386	0.180
	2	MEAN	37.612 8	37.412 8	36.957 7	36.771 7
		S.D.	0.409	0.394	0.209	0.179
	3	MEAN	38.187 8	39.100 8	38.243 7	37.600 7
		S.D.	0.318	0.472	0.932	0.645
	4	MEAN	38.750 8	38.850 8	38.214 7	37.071 7
		S.D.	0.338	0.278	0.527	0.330
	5	MEAN	36.937 8	37.112 8	37.543 7	37.557 7
		S.D.	0.414	0.300	0.331	0.299
Day 66	1	MEAN	37.725 8	37.537 8	37.300 7	37.614 7
		S.D.	0.504	0.320	0.163	0.157
	2	MEAN	37.350 8	37.425 8	36.943 7	36.629 7
		S.D.	0.434	0.388	0.140	0.236
	3	MEAN	36.775 8	38.825 8	38.357 7	37.829 7
		S.D.	0.175	0.459	0.936	0.571
	4	MEAN	37.112 8	38.825 8	39.657 7	39.371 7
		S.D.	0.242	0.358	0.374	0.496
	5	MEAN	37.812 8	37.350 8	37.714 7	38.429 7
		S.D.	0.391	0.463	0.434	0.335

	TIME	GROUP	1	2	3	4
Day 67	1	MEAN	37.612 8	37.338 8	37.214 7	37.186 7
		S.D.	0.348	0.358	0.248	0.157
	2	MEAN	37.637 8	37.537 8	37.257 7	37.443 7
		S.D.	0.463	0.220	0.326	0.461
	3	MEAN	37.137 8	39.250 8	39.157 7	39.014 7
		S.D.	0.370	0.256	0.538	0.453
	4	MEAN	37.325 8	38.762 8	39.186 7	39.496 7
		S.D.	0.427	0.307	0.372	0.248
	5[2]	MEAN	37.775 8	37.275 8	37.471 7	37.886 7
		S.D.	0.373	0.301	0.229	0.422
Day 68	1	MEAN	37.887 8	37.687 8	37.014 7	37.614 7
		S.D.	0.488	0.398	0.219	0.313
	2	MEAN	37.975 8	37.650 8	37.029 7	37.171 7
		S.D.	0.483	0.393	0.269	0.403
	3	MEAN	37.125 8	39.025 8	38.929 7	38.800 7
		S.D.	0.225	0.623	0.512	0.361
	4	MEAN	37.387 8	38.837 8	39.500 7	39.586 7
		S.D.	0.360	0.325	0.283	0.488
	5	MEAN	37.437 8	37.212 8	37.529 7	38.029 7
		S.D.	0.396	0.336	0.544	0.605
Day 69	1	MEAN	37.600 8	37.437 8	37.386 7	37.429 7
		S.D.	0.466	0.463	0.267	0.275
	2	MEAN	37.775 8	37.725 8	36.971 7	37.200 7
		S.D.	0.399	0.266	0.111	0.483
	3	MEAN	37.012 8	39.025 8	39.114 7	39.000 7
		S.D.	0.173	0.504	0.426	0.365
	4	MEAN	37.062 8	38.750 8	39.371 7	39.471 7
		S.D.	0.207	0.499	0.250	0.325
	5	MEAN	37.800 8	37.237 8	37.629 7	37.857 7
		S.D.	0.665	0.410	0.519	0.541

	TIME	GROUP	1	2	3	4
Day 70	1	MEAN	37.025 8	37.062 8	37.057 7	37.143 7
		S.D.	0.315	0.329	0.207	0.181
	2	MEAN	38.112 8	37.787 8	37.114 7	36.871 7
		S.D.	0.442	0.364	0.241	0.588
	3	MEAN	37.150 8	39.237 8	38.843 7	38.500 7
		S.D.	0.214	0.362	0.583	0.529
	4	MEAN	37.112 8	38.950 8	39.757 7	39.443 7
		S.D.	0.210	0.177	0.237	0.435
	5	MEAN	37.462 8	37.000 8	37.157 7	37.943 7
		S.D.	0.612	0.262	0.244	0.594
Day 71	1	MEAN	36.987 8	37.225 8	37.000 7	37.214 7
		S.D.	0.253	0.315	0.289	0.430
	2	MEAN	37.725 8	37.750 8	37.486 7	37.543 7
		S.D.	0.255	0.214	0.157	0.237
	3	MEAN	38.287 8	39.162 8	38.500 7	37.700 7
		S.D.	0.426	0.267	0.600	0.548
	4	MEAN	38.487 8	38.687 8	37.700 7	37.443 7
		S.D.	0.525	0.426	0.458	0.215
	5	MEAN	37.012 8	37.162 8	37.557 7	37.843 7
		S.D.	0.210	0.169	0.435	0.251
Day 72	1	MEAN	37.087 8	37.575 8	37.229 7	37.686 7
		S.D.	0.295	0.287	0.150	0.219
	2	MEAN	37.850 8	37.662 8	37.229 7	36.714 7
		S.D.	0.646	0.414	0.293	0.254
	3	MEAN	37.350 8	39.300 8	39.229 7	38.286 7
		S.D.	0.513	0.273	0.556	0.339
	4	MEAN	37.250 8	38.987 8	39.843 7	39.567 7
		S.D.	0.414	0.223	0.461	0.522
	5	MEAN	37.612 8	37.137 8	37.700 7	38.600 7
		S.D.	0.786	0.262	0.542	0.443

	TIME	GROUP	1	2	3	4
Day 73	1	MEAN	37.362 8	37.512 8	37.000 7	37.357 7
		S.D.	0.513	0.412	0.183	0.447
	2	MEAN	37.775 8	37.675 8	37.357 7	37.900 7
		S.D.	0.333	0.271	0.331	0.648
	3	MEAN	37.100 8	39.325 8	39.343 7	39.200 7
		S.D.	0.278	0.345	0.629	0.365
	4	MEAN	37.550 8	38.900 8	39.543 7	39.343 7
		S.D.	0.220	0.342	0.503	0.559
	5	MEAN	37.687 8	37.050 8	37.314 7	38.129 7
		S.D.	0.449	0.273	0.501	0.613
Day 74	1	MEAN	37.237 8	37.350 8	37.171 7	37.271 7
		S.D.	0.518	0.334	0.489	0.320
	2	MEAN	37.812 8	37.675 8	37.057 7	37.157 7
		S.D.	0.470	0.406	0.382	0.678
	3	MEAN	36.987 8	39.112 8	38.886 7	38.929 7
		S.D.	0.327	0.508	0.505	0.435
	4	MEAN	37.187 8	38.687 8	39.243 7	39.243 7
		S.D.	0.336	0.217	0.315	0.282
	5	MEAN	37.775 8	37.475 8	37.614 7	37.786 7
		S.D.	0.568	0.623	0.372	0.453
Day 75	1	MEAN	37.287 8	37.575 8	37.186 7	37.400 7
		S.D.	0.409	0.427	0.234	0.311
	2	MEAN	38.187 8	37.687 8	37.186 7	37.300 7
		S.D.	0.223	0.340	0.372	0.458
	3	MEAN	37.162 8	39.112 8	38.714 7	38.629 7
		S.D.	0.277	0.356	0.324	0.718
	4	MEAN	37.062 8	38.762 8	39.543 7	39.271 7
		S.D.	0.381	0.378	0.215	0.298
	5	MEAN	37.700 8	37.187 8	37.371 7	37.829 7
		S.D.	0.605	0.253	0.442	0.668

	TIME	GROUP	1	2	3	4
Day 76	1	MEAN	37.312 8	37.737 8	37.314 7	37.114 7
		S.D.	0.603	0.407	0.219	0.981
	2	MEAN	37.862 8	37.488 8	37.100 7	36.686 7
		S.D.	0.510	0.497	0.342	0.318
	3	MEAN	36.963 8	39.162 8	38.743 7	38.400 7
		S.D.	0.192	0.277	0.207	0.396
	4	MEAN	37.212 8	38.888 8	39.500 7	39.286 7
		S.D.	0.383	0.270	0.289	0.302
	5	MEAN	37.650 8	37.125 8	37.451 7	37.957 7
		S.D.	0.581	0.301	0.678	0.526
Day 77	1	MEAN	37.050 8	37.162 8	37.086 7	37.171 7
		S.D.	0.499	0.385	0.445	0.368
	2	MEAN	37.937 8	37.725 8	37.200 7	36.857 7
		S.D.	0.481	0.324	0.294	0.360
	3	MEAN	36.887 8	39.000 8	39.100 7	38.300 7
		S.D.	0.230	0.729	0.443	0.420
	4	MEAN	37.225 8	38.775 8	39.500 7	39.286 7
		S.D.	0.354	0.354	0.208	0.177
	5	MEAN	37.312 8	37.237 8	37.200 7	37.757 7
		S.D.	0.530	0.553	0.283	0.506
Day 78	1	MEAN	37.087 8	37.112 8	37.314 7	37.214 7
		S.D.	0.432	0.203	0.219	0.353
	2	MEAN	37.625 8	37.575 8	37.000 7	36.729 7
		S.D.	0.381	0.311	0.173	0.263
	3	MEAN	36.950 8	39.038 8	38.557 7	38.329 7
		S.D.	0.267	0.498	0.973	0.315
	4	MEAN	37.262 8	38.813 8	39.214 7	39.314 7
		S.D.	0.410	0.309	0.596	0.261
	5	MEAN	37.700 8	36.987 8	37.543 7	37.471 7
		S.D.	0.434	0.327	0.299	0.596

	TIME	GROUP	1	2	3	4
Day 79	1	MEAN	36.950 8	37.112 8	37.157 7	37.329 7
		S.D.	0.278	0.356	0.310	0.281
	2	MEAN	37.750 8	37.712 8	37.043 7	36.814 7
		S.D.	0.273	0.275	0.360	0.177
	3	MEAN	37.100 8	37.287 8	36.886 7	36.757 7
		S.D.	0.283	0.318	0.204	0.172
	4	MEAN	37.012 8	37.187 8	37.014 7	36.700 7
		S.D.	0.164	0.318	0.267	0.183
	5	MEAN	37.737 8	38.063 8	37.800 7	37.814 7
		S.D.	0.607	0.302	0.370	0.344
Day 80	1	MEAN	37.087 8	37.125 8	37.029 7	37.443 7
		S.D.	0.449	0.413	0.229	0.374
	2	MEAN	37.987 8	37.912 8	37.371 7	36.971 7
		S.D.	0.398	0.327	0.180	0.304
	3	MEAN	36.987 8	39.338 8	39.186 7	38.257 7
		S.D.	0.323	0.342	0.609	0.864
	4	MEAN	37.137 8	39.050 8	39.957 7	39.357 7
		S.D.	0.410	0.131	0.299	0.522
	5	MEAN	37.362 8	37.037 8	37.943 7	38.500 7
		S.D.	0.542	0.226	0.583	0.252
Day 81	1	MEAN	37.038 8	37.375 8	37.400 7	37.086 7
		S.D.	0.550	0.282	0.271	0.241
	2	MEAN	37.887 8	37.700 8	37.571 7	37.729 7
		S.D.	0.455	0.256	0.229	0.668
	3	MEAN	36.925 8	39.100 8	39.486 7	38.886 7
		S.D.	0.413	0.529	0.449	0.576
	4	MEAN	37.250 8	38.712 8	39.700 7	39.486 7
		S.D.	0.421	0.344	0.265	0.549
	5	MEAN	37.325 8	37.137 8	37.086 7	37.729 7
		S.D.	0.761	0.151	0.344	0.475

	TIME	GROUP	1	2	3	
Day 82	1	MEAN	37.387 8	37.725 8	37.329 7	37.257 7
		S.D.	0.730	0.337	0.275	0.374
	2	MEAN	38.037 8	37.425 8	37.043 7	37.129 7
		S.D.	0.256	0.468	0.140	0.547
	3	MEAN	37.137 8	38.625 8	38.443 7	38.429 7
		S.D.	0.272	0.578	0.648	0.697
	4	MEAN	37.437 8	38.862 8	39.186 7	39.543 7
		S.D.	0.475	0.444	0.376	0.369
	5	MEAN	37.187 8	37.225 8	37.829 7	37.800 7
		S.D.	0.522	0.403	0.486	0.523
Day 83	1	MEAN	37.225 8	37.287 8	37.071 7	36.957 7
		S.D.	0.465	0.452	0.320	0.391
	2	MEAN	37.962 8	37.725 8	37.286 7	37.300 7
		S.D.	0.540	0.403	0.382	0.462
	3	MEAN	37.125 8	38.738 8	38.614 7	38.686 7
		S.D.	0.459	0.354	0.654	0.456
	4	MEAN	37.062 8	38.600 8	38.971 7	39.329 7
		S.D.	0.437	0.472	0.355	0.399
	5	MEAN	37.412 8	37.000 8	37.300 7	37.371 7
		S.D.	0.551	0.262	0.383	0.678
Day 84	1	MEAN	36.862 8	37.212 8	36.886 7	36.957 7
		S.D.	0.239	0.557	0.177	0.299
	2	MEAN	37.600 8	37.500 8	37.043 7	36.671 7
		S.D.	0.283	0.389	0.288	0.263
	3	MEAN	36.912 8	39.137 8	38.843 7	38.257 7
		S.D.	0.340	0.385	0.458	0.331
	4	MEAN	37.450 8	38.963 8	39.471 7	39.614 7
		S.D.	0.472	0.239	0.359	0.334
	5	MEAN	37.262 8	36.925 8	37.300 7	37.886 7
		S.D.	0.518	0.205	0.258	0.463

	TIME	GROUP	1	2	3	4
Day 85	1	MEAN	37.237 8	37.300 8	37.286 7	37.114 7
		S.D.	0.447	0.207	0.363	0.254
	2	MEAN	37.637 8	37.812 8	37.300 7	37.386 7
		S.D.	0.487	0.270	0.271	0.469
	3	MEAN	37.100 8	37.150 8	37.043 7	37.171 7
		S.D.	0.370	0.193	0.244	0.350
	4	MEAN	37.050 8	37.350 8	36.971 7	37.386 7
		S.D.	0.382	0.251	0.250	0.308
	5	MEAN	37.512 8	37.700 8	37.714 7	37.614 7
		S.D.	0.577	0.428	0.348	0.291
Day 86	1	MEAN	37.237 8	37.287 8	37.529 7	37.571 7
		S.D.	0.490	0.391	0.368	0.275
	2	MEAN	38.050 8	37.675 8	37.514 7	37.500 7
		S.D.	0.278	0.410	0.234	0.416
	3	MEAN	37.200 8	37.362 8	37.229 7	37.071 7
		S.D.	0.563	0.463	0.150	0.565
	4	MEAN	36.950 8	37.425 8	37.314 7	37.271 7
		S.D.	0.239	0.354	0.157	0.386
	5	MEAN	37.075 8	37.962 8	37.971 7	37.800 7
		S.D.	0.636	0.350	0.243	0.383
Day 87	1	MEAN	37.037 8	37.150 8	37.529 7	37.357 7
		S.D.	0.407	0.513	0.373	0.305
	2	MEAN	38.112 8	37.962 8	37.786 7	37.429 7
		S.D.	0.376	0.302	0.313	0.345
	3	MEAN	37.950 8	37.575 8	37.486 7	37.571 7
		S.D.	0.571	0.468	0.308	0.250
	4	MEAN	37.212 8	37.250 8	37.386 7	37.386 7
		S.D.	0.217	0.267	0.157	0.135
	5	MEAN	37.325 8	37.462 8	37.900 7	37.757 7
		S.D.	0.807	0.226	0.476	0.215

	TIME	GROUP	1	2	3	4
Day 88	1	MEAN	37.263 8	37.337 8	37.657 7	37.314 7
		S.D.	0.689	0.434	0.424	0.402
	2	MEAN	37.825 8	37.762 8	37.800 7	37.271 7
		S.D.	0.575	0.542	0.208	0.355
	3	MEAN	37.075 8	37.662 8	37.843 7	37.400 7
		S.D.	0.365	0.358	0.190	0.306
	4	MEAN	36.963 8	37.325 8	37.429 7	37.186 7
		S.D.	0.169	0.198	0.250	0.157
	5	MEAN	37.025 8	37.387 8	37.729 7	37.500 7
		S.D.	0.504	0.300	0.325	0.191
Day 89	1	MEAN	37.200 8	36.912 8	37.300 7	37.200 7
		S.D.	0.490	0.125	0.370	0.379
	2	MEAN	37.837 8	37.937 8	37.900 7	37.443 7
		S.D.	0.338	0.403	0.271	0.346
	3	MEAN	37.162 8	37.487 8	37.300 7	37.257 7
		S.D.	0.498	0.402	0.141	0.331
	4	MEAN	37.025 8	37.225 8	37.457 7	37.386 7
		S.D.	0.301	0.311	0.483	0.177
	5	MEAN	37.125 8	37.362 8	37.486 7	37.314 7
		S.D.	0.550	0.311	0.324	0.339
Day 90	1	MEAN	37.287 8	37.175 8	37.500 7	37.157 7
		S.D.	0.666	0.477	0.580	0.364
	2	MEAN	37.862 8	37.812 8	37.814 7	37.529 7
		S.D.	0.334	0.380	0.367	0.335
	3	MEAN	37.012 8	37.425 8	37.343 7	37.457 7
		S.D.	0.436	0.440	0.341	0.496
	4	MEAN	36.987 8	37.050 8	37.114 7	37.157 7
		S.D.	0.136	0.220	0.248	0.336
	5	MEAN	37.225 8	37.300 8	37.343 7	37.329 7
		S.D.	0.462	0.370	0.355	0.446

	TIME	GROUP	1	2	3	4
Day 91	1	MEAN	37.250 8	37.325 8	37.386 7	37.171 7
		S.D.	0.540	0.410	0.576	0.293
	2	MEAN	37.800 8	37.800 8	37.729 7	37.729 7
		S.D.	0.550	0.605	0.556	0.489
	3	MEAN	37.237 8	37.800 8	37.457 7	37.286 7
		S.D.	0.540	0.478	0.412	0.393
	4	MEAN	36.850 8	36.925 8	37.000 7	36.914 7
		S.D.	0.131	0.205	0.115	0.291
	5	MEAN	36.962 8	37.237 8	37.486 7	37.186 7
		S.D.	0.338	0.434	0.261	0.329
Day 92	1	MEAN	37.325 8	37.300 8	37.186 7	37.557 7
		S.D.	0.618	0.396	0.667	0.577
	2	MEAN	37.562 8	38.075 8	37.929 7	37.571 7
		S.D.	0.542	0.647	0.457	0.386
	3	MEAN	37.375 8	37.475 8	37.271 7	37.243 7
		S.D.	0.661	0.306	0.287	0.374
	4	MEAN	37.075 8	37.212 8	37.314 7	37.043 7
		S.D.	0.287	0.270	0.324	0.399
	5	MEAN	37.287 8	37.637 8	37.943 7	37.071 7
		S.D.	0.436	0.487	0.382	0.198
Day 93	1	MEAN	37.212 8	37.037 8	37.214 7	37.286 7
		S.D.	0.557	0.358	0.581	0.393
	2	MEAN	37.863 8	38.025 8	37.671 7	37.614 7
		S.D.	0.665	0.392	0.496	0.590
	3	MEAN	37.175 8	37.525 8	37.657 7	37.529 7
		S.D.	0.349	0.369	0.391	0.629
	4	MEAN	37.175 8	37.312 8	37.357 7	37.271 7
		S.D.	0.417	0.230	0.127	0.275
	5	MEAN	37.450 8	37.462 8	37.786 7	37.343 7
		S.D.	0.614	0.424	0.445	0.496

	TIME	GROUP	1	2	3	4
Day 94	1	MEAN	37.125 8	37.137 8	37.229 7	37.043 7
		S.D.	0.420	0.320	0.350	0.264
	2	MEAN	37.625 8	37.837 8	37.800 7	37.729 7
		S.D.	0.495	0.358	0.396	0.446
	3	MEAN	37.175 8	37.575 8	37.486 7	37.371 7
		S.D.	0.362	0.486	0.438	0.275
	4	MEAN	36.987 8	37.312 8	37.143 7	37.157 7
		S.D.	0.203	0.485	0.223	0.321
	5	MEAN	37.125 8	37.387 8	37.386 7	37.186 7
		S.D.	0.139	0.470	0.430	0.248
Day 95	1	MEAN	37.350 8	37.162 8	37.343 7	37.143 7
		S.D.	0.711	0.350	0.550	0.378
	2	MEAN	38.625 8	38.650 8	38.529 7	38.486 7
		S.D.	0.450	0.227	0.304	0.273
	3	MEAN	37.912 8	38.300 8	38.329 7	38.343 7
		S.D.	0.624	0.359	0.263	0.341
	4	MEAN	37.475 8	37.975 8	38.486 7	38.071 7
		S.D.	0.680	0.580	0.318	0.236
	5	MEAN	37.100 8	37.062 8	37.129 7	37.143 7
		S.D.	0.325	0.177	0.189	0.519
Day 96	1	MEAN	37.300 8	37.362 8	37.071 7	37.300 7
		S.D.	0.550	0.609	0.442	0.507
	2	MEAN	38.350 8	38.375 8	38.257 7	38.186 7
		S.D.	0.351	0.373	0.207	0.195
	3	MEAN	37.700 8	38.187 8	38.171 7	38.029 7
		S.D.	0.325	0.387	0.206	0.256
	4	MEAN	37.375 8	38.225 8	38.243 7	37.971 7
		S.D.	0.446	0.437	0.305	0.281
	5	MEAN	37.662 8	37.900 8	37.971 7	37.914 7
		S.D.	0.646	0.545	0.435	0.324

TIME	GROUP	1	2	3	4
Day 97	1 MEAN	37.275 8	37.275 8	37.529 7	37.057 7
	S.D.	0.523	0.534	0.634	0.315
	2 MEAN	37.937 8	38.262 8	38.200 7	38.157 7
	S.D.	0.431	0.302	0.271	0.336
	3 MEAN	36.912 8	37.587 8	37.400 7	37.357 7
	S.D.	0.300	0.533	0.486	0.310
	4 MEAN	37.212 8	37.937 8	38.086 7	37.943 7
	S.D.	0.352	0.605	0.515	0.562
	5 MEAN	37.800 8	38.100 8	38.414 7	38.257 7
	S.D.	0.713	0.586	0.297	0.571
Day 98	1 MEAN	36.975 8	36.825 8	37.200 7	36.800 7
	S.D.	0.381	0.249	0.432	0.224
	2 MEAN	37.750 8	38.112 8	38.014 7	37.957 7
	S.D.	0.389	0.259	0.267	0.257
	3 MEAN	37.512 8	37.887 8	38.100 7	37.886 7
	S.D.	0.461	0.264	0.163	0.329
	4 MEAN	37.687 8	38.175 8	38.300 7	38.143 7
	S.D.	0.708	0.406	0.216	0.264
	5 MEAN	37.250 8	37.537 8	37.814 7	37.914 7
	S.D.	0.568	0.362	0.527	0.474
Day 99	1 MEAN	37.150 8	37.387 8	37.343 7	37.129 7
	S.D.	0.524	0.376	0.503	0.468
	2 MEAN	37.787 8	38.063 8	38.043 7	37.829 7
	S.D.	0.439	0.283	0.288	0.457
	3 MEAN	37.112 8	37.925 8	38.000 7	37.929 7
	S.D.	0.318	0.260	0.163	0.206
	4 MEAN	37.387 8	37.962 8	38.071 7	37.943 7
	S.D.	0.494	0.478	0.138	0.244
	5 MEAN	38.012 8	38.000 8	38.043 7	37.771 7
	S.D.	0.405	0.493	0.215	0.377

TIME	GROUP	1	2	3	4
Day 100	1 MEAN	37.025 8	36.888 8	37.371 7	36.957 7
	S.D.	0.620	0.467	0.315	0.583
	2 MEAN	37.375 8	37.500 8	37.743 7	37.543 7
	S.D.	0.406	0.400	0.435	0.465
	3 MEAN	37.412 8	37.637 8	37.814 7	37.586 7
	S.D.	0.533	0.316	0.402	0.353
	4 MEAN	37.212 8	37.350 8	37.743 7	37.543 7
	S.D.	0.242	0.267	0.435	0.321
	5 MEAN	37.712 8	38.412 8	38.271 7	37.971 7
	S.D.	0.476	0.217	0.364	0.335
Day 120	1 MEAN	37.387 8	37.225 8	37.471 7	37.643 7
	S.D.	0.394	0.392	0.727	0.550
	2 MEAN	38.225 8	38.025 8	38.300 7	38.586 7
	S.D.	0.526	0.392	0.379	0.329
	3 MEAN	37.762 8	37.912 8	38.000 7	37.929 7
	S.D.	0.410	0.479	0.606	0.594
	4 MEAN	37.337 8	37.237 8	37.386 7	37.229 7
	S.D.	0.277	0.325	0.573	0.377
	5 MEAN	37.225 8	37.250 8	37.200 7	37.514 7
	S.D.	0.443	0.501	0.277	0.367
Day 121	1 MEAN	37.975 8	38.275 8	38.186 7	38.029 7
	S.D.	0.876	0.358	0.530	0.660
	2 MEAN	38.575 8	38.637 8	38.529 7	38.600 7
	S.D.	0.282	0.185	0.373	0.163
	3 MEAN	37.862 8	38.250 8	38.300 7	38.443 7
	S.D.	0.457	0.214	0.374	0.190
	4 MEAN	37.700 8	38.037 8	38.257 7	38.114 7
	S.D.	0.685	0.635	0.364	0.273
	5 MEAN	37.012 8	37.363 8	37.114 7	37.300 7
	S.D.	0.242	0.521	0.195	0.383

	TIME	GROUP	1	2	3	4
Day 122	1	MEAN	37.337 8	37.562 8	37.800 7	37.671 7
		S.D.	0.374	0.571	0.864	0.522
	2	MEAN	37.900 8	38.288 8	38.400 7	38.129 7
		S.D.	0.532	0.340	0.361	0.206
	3	MEAN	37.713 8	38.138 8	38.029 7	38.014 7
		S.D.	0.584	0.469	0.535	0.679
	4	MEAN	37.525 8	37.637 8	37.543 7	37.457 7
		S.D.	0.381	0.424	0.346	0.496
	5	MEAN	38.012 8	37.875 8	37.900 7	38.243 7
		S.D.	0.700	0.800	0.714	0.772